THE ANALYST

PROCEEDINGS OF THE SOCIETY FOR ANALYTICAL CHEMISTRY

NEW MEMBERS

Ordinary Members

Michael John Bowley, B.Sc. (Bris.); Thomas Arthur Brock, B.Sc. (Lond.), F.R.I.C., A.F.Inst.Pet.; Roy George Clark, B.Sc. (Lond.), A.R.I.C.; Ronald Maurice Cooper, M.Sc. (Lond.); Edwin Fletcher; Joan Meakin, B.Sc. (Lond.); Frend John Miner, B.A. (Colorado), M.S., Ph.D. (Oregon); Howard Antrobus Moule, B.Sc. (Lond.), A.R.I.C.; Howard Alfred Nicholls, A.R.I.C.; Kenneth Charles Nutt, B.Sc. (Lond.); John Wilson Ogleby, B.Sc. (Dunelm.); Joseph Howard Oldfield, F.R.I.C.; Ernest Walter Peacock, B.Sc. (Lond.); Richard Redvers Scott, B.A. (Cantab.); Brian William Stannard, B.Sc. (Lon.), A.R.I.C.; Bernard Thomas Dudley Sully, B.Sc., Ph.D. (Lond.), A.R.C.S., F.R.I.C.

Junior Members

Montague John Giles; Margaret Davidson Macuean, B.Sc. (Glas.); John Lawrence Roberts; Sydney Williams.

DEATH

We record with regret the death of

David George Allen
Geoffrey Alfred Bracewell
Donald Ford Phillips.

NORTH OF ENGLAND SECTION

The thirty-sixth Annual General Meeting of the Section was held at 2.15 p.m on Saturday, January 28th, 1961, at the "Nag's Head Hotel," Lloyd Street, Manchester. The Chairman of the Section, Dr. J. R. Edisbury, presided. The following appointments were made for the ensuing year:—*Chairman*—Mr. J. Markland. *Vice-Chairman*—Mr. C. J. House. *Hon. Secretary and Treasurer*—Mr. B. Hulme, Ch. Goldrei, Foucard & Son Ltd., Brookfield Drive, Liverpool, 9. *Members of Committee*—Mr. R. Butler, Mr. G. B. Crump, Dr. W. Cule Davies, Mr. A. O. Jones, Mr. G. F. Longman and Mr. R. Sinar. Mr. A. A. D. Comrie and Mr. F. Dixon were appointed Hon. Auditors.

The Annual General Meeting was followed by an Ordinary Meeting of the Section, when Dr. J. R. Edisbury gave his Retiring Chairman's Address. The Chair at this meeting was taken by the new Chairman of the Section, Mr. J. Markland, B.Sc., F.R.I.C.

SCOTTISH SECTION

THE twenty-sixth Annual General Meeting of the Section was held at 6.30 p.m. on Thursday, January 26th, 1961, at the Grosvenor Restaurant, 72 Gordon Street, Glasgow, C.1. The Chair was taken by the Chairman of the Section, Mr. A. N. Harrow, A.H.-W.C., F.R.I.C. The following office bearers were elected for the forthcoming year:—*Chairman*—Mr. A. F. Williams. *Vice-Chairman*—Dr. R. A. Chalmers. *Hon. Secretary and Treasurer*—Mr. J. Brooks, Research and Development Department, Imperial Chemical Industries Ltd., Nobel Division, Stevenston, Ayrshire. *Members of Committee*—Dr. F. J. Elliott, Mr. J. K. McLellan, Mr. H. C. Moir, Mr. J. W. Murfin and Mr. S. C. Sloan. Mr. C. B. Hackett and Mr. W. J. Murray were re-appointed Hon. Auditors.

The Annual General Meeting was followed by an Ordinary Meeting of the Section, at which the Chair was taken by the retiring Chairman of the Section, Mr. A. N. Harrow, A.H.-W.C., F.R.I.C. The following paper was presented: "Chemical Research in the Electricity Supply Industry," by J. M. Ward, B.Sc., F.R.I.C.

MICROCHEMISTRY GROUP

THE twenty-eighth London Discussion Meeting of the Group was held at 6.30 p.m. on Wednesday, January 25th, 1961, at "The Feathers," Tudor Street, London, E.C.4. The Chair was taken by the Vice-Chairman of the Group, Mr. C. Whalley, B.Sc., F.R.I.C.

A discussion on "Hydrogenation Methods in Microanalysis" was opened by R. A. D. Smith.

Obituary

HUBERT THOMAS STANLEY BRITTON

HUBERT THOMAS STANLEY BRITTON died at the early age of 68 on December 30th, 1960, after a brief illness. He is survived by his son and daughter. His death is a grievous loss to analytical chemistry, the more so since he leaves much unpublished work, which it had been his intention to write up during his retirement.

Born at Kingswood, near Bristol, on April 22nd, 1892, the son of a shoemaker, he was educated at St. George's Grammar School and the Merchant Venturers' College. His entry on a scholarship to Bristol University in 1911 was almost prevented by the unfortunate death of his father in an accident, but the crisis was averted by the generosity of the people of his village, a kindness of which Britton often spoke. After graduation in 1914 and a short spell of schoolmastering, he was posted to the Aeronautics Inspection Department, and served, mostly in Wolverhampton, for the duration of the war. In 1920 he became an assistant lecturer at King's College, London, where he began the researches that led to the awards of D.Sc. (London) in 1926 and D.Sc. (Bristol) in 1934. He published eighteen papers before leaving to take up a senior D.S.I.R. award at Imperial College in 1925. On the termination of the award he suffered considerable hardship, having then a young family, through several months of unemployment. During this time he wrote a number of articles for the technical press: these later formed the basis of his "Hydrogen Ions." After a year as lecturer at Norwood Technical College, Britton moved in 1929 to the then University College of the South-West, where he remained, first as lecturer and later, from 1935, as professor, until his retirement in 1957 with the title of Professor Emeritus in the University of Exeter.

Britton's researches started with an interest in beryllium, including a very sound appraisal of its analytical chemistry (*Analyst*, 1921, **46**, 359, 437; 1922, **47**, 50), moved through phase studies with beryllium and chromium compounds, and quickly settled on a long series of studies by electrometric methods of homogeneous and heterogeneous ionic equilibria. These embraced hydrolytic precipitation (16 papers), complex acids (15 papers), complexation reactions involving weak acids (17 papers), and many other shorter series and individual papers of direct fundamental analytical interest. Very many of the physico-chemical constants emanating

from his laboratory remain unchallenged today. The work on hydrolysis and complexation, of which Britton has himself left some connected account (*Annual Reports of the Chemical Society*, **1943**, **40**, **43**), has undoubtedly paved the way to the modern unified concept of complexation reactions, which includes hydrolysis, acid - base ionisation and precipitation reactions. The bulk of this was published in the decade 1925 to 1935, and work of such difficulty and complexity required not only a very clear and wide vision, but also courage of no common order in the essay. Polymeric species such as $Be_2(OH)_2^{2+}$ were clearly in his thoughts 40 years ago, and one has only to consult modern tables of stability constants to find witness to his wisdom. Although he was much more interested in the fundamental data they would yield than in the origination of new devices, and he did not discover any new electrode systems, he was nevertheless very early in the field, and made extensive and significant contributions to the use and improvement of the hydrogen, oxygen, antimony, tungsten and glass electrodes. Conductometry was in early requisition in his work, and he pioneered the use of optical rotation in titrimetry. He had considerable interest in precipitimetric and redox titrimetry, and made important contributions to such widely diverse fields as chromium plating and the recovery of magnesium from sea water, and served on the B.S.I. committee on the standardisation of pH scales. Nearly 100 papers stand witness to his industry, with material for half as many again yet unpublished. The Britton - Robinson universal buffer is known to all, while within the covers of "Hydrogen Ions," the fourth edition of which he completed just before he retired, is to be found a wealth of information such as can seldom be encompassed in such a work, and all of it bearing the stamp of intimate personal acquaintance. For many years to come the two volumes of this book will remain indispensable in all laboratories concerned with pH and the physical chemistry of ionic solutions.

During his early years at Exeter, Britton was charged with the whole of the teaching and laboratory supervision for the Honours Degree, yet he contrived to produce "Conductometric Analysis" and the second edition of "Hydrogen Ions," to be at his most active in research, to direct the work of a research assistant and many research students, and to do much extra-mural lecturing. This prodigious industry was characteristic of the man and continued during his tenure of the Chair. He was very active on Senate, and during the war years undertook the onerous duties of Dean of the Faculty of Science, besides serving as Senior Gas Adviser for Devon and Cornwall, acting as host to evacuated departments from London, and lecturing extensively to the Forces in the area.

Subsequently he was drawn more and more from the bench and library by the demands of an expanding department, by preparations for the elevation of the College to University status and by the preliminary planning of a new building for the department. He continued vigorously to direct research, was instrumental in securing the formation of local sections of the Royal Institute of Chemistry and the Society of Chemical Industry, continued his forensic and extra-mural work, served on the Councils of our three sister Societies, and served as Chairman of Governors of the North Devon Technical College during a critical period.

Britton was a man whom to know was to like and respect. He was outstandingly human, warmhearted and friendly. A flavour of the sceptic and the rebel added piquancy to his character. Always accessible, his advice, encouragement and help were freely given to student and colleague alike, and were as freely sought and highly valued. His lively personality was demonstrated in his animated, entertaining and stimulating lectures, both to undergraduates and to audiences in the depth of the country. Ever interested in his fellow men and in affairs and with a fund of both sage and amusing reminiscences, he was a sympathetic and enlivening companion. His brief retirement was sadly clouded by anxiety over the health of his wife and by her death in 1959. Britton's sudden death was a great shock to all who knew him, particularly as he retained, outwardly at least, his full vigour and health until his final short illness, and the loss is made the greater by his inability to accomplish his objective of publishing the remainder of his work. He will long remain in the affectionate memory of the many students who passed through his hands and of all those who worked with him.

E. BISHOP

Determination of Residual Organo-phosphorus Insecticides in Foodstuffs

A Review*

By E. D. CHILWELL and G. S. HARTLEY

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Delnav
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I. Introduction

The increasing use of toxic pesticides in agriculture has led to increasing awareness in government and medical circles of the problem of safe-guarding the consumer. Many countries, including the United States of America and the U.S.S.R., have introduced rigid legislation requiring detailed examination of all aspects of the potential hazard before new insecticides can be approved for specific usages. At the stage of enquiry and for purposes of implementing legislation, analytical methods are needed for determining residual contamination of foodstuffs, often in such trace amounts as to present a major technical problem for the analyst.

The need for methods of determining residues is not confined to organo-phosphorus compounds, but, for several reasons, it came into prominence along with their development. First, these compounds are similar in chemical structure and mode of action to compounds developed for chemical warfare and therefore are of notoriously high toxicity to mammals. Secondly, the first widely used non-phosphorus synthetic insecticides, DDT and hexachlorocyclohexane (BHC) were brought into use in the latter stages of the second World War, when investigation of minor hazards to the civilian population could not command a very high priority, and, being of low acute toxicity, they were accepted and established before much attention was paid to effects of prolonged low-level ingestion.

Thirdly, they include the first organic systemic insecticides, capable of migration in the transport system of living plants; such compounds were naturally held to constitute a greater danger than those remaining on the external surfaces. It is in fact not necessarily true that systemic insecticides are more persistent than superficial ones, because the latter can to a large extent be dissolved in the leaf cuticle where they are resistant to aqueous washing, whereas the former, though inaccessible to washing, are in closer contact with the more chemically active plant tissues and so more subject to chemical destruction.

* Reprints of this paper will be available shortly. For details, please see p. 207.

Sections III and IV of this review are largely relevant only to organo-phosphorus compounds, but so much of what we have to say in section II, dealing with extraction procedures, is of general validity that it has been considered advisable in this section to draw on the literature of extraction generally.

The procedures for determining residues are largely dictated by the technical problems involved. Such problems differ in the requirements of (1) the manufacturer of a new pesticide chemical called upon to submit evidence on the safety of his product to the consumer, (2) the analyst called upon to decide whether or not a sample of foodstuff contains a residue of a particular insecticide in excess of a given limit and (3) the public analyst who is asked if a foodstuff of unknown history contains an undesirable amount of any insecticide residue.

The requirements of residue analysis for clearance under the Voluntary Notification Scheme in the United Kingdom have been described[1]; they are the determination of the amount of the chemical applied and of any toxic derivative of that chemical remaining in the crop at harvest. The research analyst is usually, or ideally, concerned with the organisation of experiments to prove the amount and nature of the residue present. At all times he is confident that no other insecticide could be present in the samples examined, and untreated samples of the crop are available. Methods do not need to be specific; in fact methods of broader spectrum are desirable because they are more likely to include metabolites of the chemical applied. The methods available at this stage can include many that are not practicable for the analyst concerned with a commercial crop. The use of radio-labelled insecticides permits a fuller examination of the errors in a final procedure than is possible by classical chemical methods. Their use is particularly valuable in the more biochemical aspects of the investigation, such as identifying products derived by plant chemistry.

The second problem is that of the analyst required to determine accurately a named chemical in a foodstuff and certify that the amount present does not exceed a given limit. This need is for recognised standard methods of analysis that have been proved accurate by collaborative investigation in a number of different laboratories. Such collaborative examinations of promising methods are carried out by the Association of Official Agricultural Chemists in North America and by joint sub-committees of Government Departments, Industry and the Society for Analytical Chemistry in this country.

The problem of the analyst presented with a sample of a foodstuff to examine for any possible insecticide residue is much more difficult. There is no close relation between amount present and consumer hazard unless the insecticide is known. Therefore, in the absence of knowledge of the insecticides present, the result would have to be interpreted as though the whole contamination were due to the most toxic insecticide in use. This would lead to unjustifiable condemnation of products in fact safely consumable. The problem could only be met in the chemical laboratory by working out some quick scheme of qualitative analysis to be applied before proceeding to quantitative assessment. The only serious attention yet given to this aspect of the subject is the possibility of identification in two-dimensional paper chromatograms,[2] so far most completely worked out for chlorinated hydrocarbons.

An alternative solution would be a method of determination in which "toxicity" is directly measured—*i.e.*, a bioassay—and a Sub-Committee of the Analytical Methods Committee of the Society for Analytical Chemistry is at present considering the practicability of this. The proposal meets several difficulties. First, analytical laboratories are not usually equipped for such methods. Secondly, they are expensive if mammals are used, or, if insects are used, the results are not necessarily more significant than those from a general chemical method, since it is an obvious and desirable tendency in research into new insecticides to bring forward those with the highest ratio of insect to mammalian toxicity.

Lastly, the analyst is not usually called upon to find an amount of insecticide constituting a serious risk of death to the consumer, but rather for an amount that might produce marginal ill-health. He must therefore concentrate the insecticide so that acute toxicity can be demonstrated, without concentrating at the same time any natural toxicants, or he must use some physiological response well short of death. The former demands knowledge of the insecticides present and does not therefore use the potential advantage of bioassay. The latter is perhaps possible at present for organo-phosphorus compounds, owing to their producing measurable reduction in blood cholinesterase level long before clinical symptoms are discernible. Feeding tests on mammals, with maximal amounts of the product, and

determining blood cholinesterase might provide an answer to the general question "is there any significant contamination by any organo-phosphorus compound?" The method will, however, probably always be considered too lengthy to meet the requirements for clearance of perishable foodstuffs.

A half-way stage between bioassay and chemical determination is assessment of cholinesterase inactivation *in vitro*. The enzyme is readily accessible and its hydrolysis gives rise to easily measured acid. Applied to residue analysis the method is not particularly satisfactory for those compounds (the extreme example of which is schradan) that do not inhibit the enzyme *in vitro*, but only after conversion in the tissues of the animal. Again, if used after a broad method of separation, it is subject to interference by those natural products (not very widespread) that also inactivate the enzyme or by natural esters that can increase the acid liberated by the not very specific enzyme.

To date, in fact, no satisfactory solution has been found to the problem of devising a method at once non-specific, reliable and significant.

II. Extraction of the insecticide

Before the residual insecticide can be subjected to a selective concentration process that isolates it adequately for the method of determination to be used, it must be extracted from the vegetable tissue into a convenient form of matter. This is almost always a solution in water or organic solvent, but occasionally processes approximating to direct distillation have been used.

The extraction procedure is desirably itself selective to some extent, when this is consistent with its being adequately complete. Thus, stable and almost water-insoluble insecticides, particularly when crystalline, do not penetrate deeply into the aqueous internal tissues of plants, although they may diffuse into the swellable polymeric cuticle substance and the more oily of the soluble associated substances. The residue can then be mostly extracted by a short period of washing of the intact plant or fruit in an effective organic solvent, a process usually referred to as stripping. It is not safe to assume that a short period of washing is adequate, nor that a treatment found adequate for leaves or fruits of one species is adequate for another, since there is great variation in cuticle thickness and composition. Experimental proof of adequate extraction must always be obtained (see below).

Surface extraction procedure on more or less intact plant organs is desirable, when applicable, because the inclusion in the extract of large amounts of internal plant compounds that may have interfering properties is largely avoided. Such interference may be chemical, in the final process of determination, or physical, in that troublesome emulsions or precipitations may arise during the "clean-up" procedure.

Washing procedures can be more or less drastic and deep-searching in their action, according to the solvent chosen and the state of the plant tissues. Washing of fresh intact leaves in hydrocarbon solvents removes little of the naturally occurring soluble substances except those found on or in the cuticle, even when carried on for periods of several hours. If the leaves are considerably desiccated before being washed or if washing of fresh leaves is carried out with a water-soluble solvent, much more material is dissolved from within the tissues, presumably because the various membrane barriers lose their resistance when their texture is altered by removal of water. Extraction with hot alcohol in a Soxhlet apparatus will eventually remove practically everything except the skeletal substances, but the product of such extraction necessitates troublesome cleaning up.

When extraction from within the tissues is necessary, the vegetable material is generally reduced to a fine "brei" by violent mechanical agitation in a Waring Blendor or similar machine. If the insecticide to be extracted is reasonably soluble in water (and deep penetration in the tissues does not occur unless it is), maceration is usually carried out in water and the insecticide extracted from the water by a more suitable, and only incompletely miscible, solvent, usually chloroform. The extraction is best carried out after filtration, as the emulsions then formed are less intractable and can usually be broken by centrifugation. Sometimes, however, maceration directly in an organic solvent is preferred; for example Jones and Riddick[3] macerate plant tissue with hexane and extract the hexane filtrate with acetonitrile.

A procedure that has much to commend it aims at complete extraction of soluble small-molecule substances while largely avoiding the physically troublesome semi-solid and colloidal constituents. Chopped, but not macerated, tissue is extracted with a good, but not water-

miscible, solvent—*e.g.*, benzene or chloroform—in the presence of a desiccating agent—*e.g.*, anhydrous sodium sulphate. The restricted permeability of most membranes is lost by the desiccation and more direct contact of fatty globules and solvent permitted, but the reduced mechanical damage as compared with maceration restricts the passage of solid debris into the extractant. The tissue itself in fact acts as a filter. The dessicant and chopped tissue are mixed in a column and slowly percolated.[4]

The proof of complete extraction is not easy, but is an essential step in making sure that the whole procedure gives a significant result. Steps taken for such proof are—

(1) Carrying out extractions for different periods of time, it being assumed in interpreting the results that complete extraction is obtained when not appreciably more is extracted by a longer process.

(2) Comparing the proposed method of extraction with one assumed to be complete —such as exhaustive extraction with hot alcohol in a Soxhlet apparatus.

(3) Quantitative introduction of insecticide, preferably carrying a radio label, by the "cut stalk" technique if systemic or by painting on if non-systemic, and comparing the amount extracted with that applied.

None of these is free from objection. Step (1) would be invalidated if a proportion of insecticide is located in cells from which extraction is difficult, although most of it is easily accessible. When cold hydrocarbon solvents are used to extract natural waxes and fats from turgid leaves, the extracuticular matter is completely dissolved in a few minutes (mostly in seconds). Unpublished work in our laboratories has shown that a subsequent enormous slowing down is not evidence of complete extraction. A further similar amount of hydrocarbon-soluble substances is obtained when water is removed. The substances here are qualitatively different, but an extraneous substance, which *had been allowed time* to distribute, would doubtless show similar behaviour.

There is a possible error in the opposite sense in step (2), since such drastic extraction may decompose a significant amount of the insecticide. Among the organo-phosphorus compounds, probably only schradan is sufficiently stable for this not to occur.

In step (3), rapid determination is necessary unless so much decomposition is to be allowed that a quantitative balance cannot be expected.[5] With rapid determination, a good balance cannot be taken as evidential because there has not been time for the insecticide to move out of the accessible sites of application into less accessible sites.

Step (1) at least should always be carried out, but additional confirmation is desirable. This is best obtained by animal feeding experiments run side by side with the proposed analysis.[6] These give a direct check on the significance of the results for the purpose for which they are obtained. Not only does a successful check in this way rule out the possibility of insecticide being occluded in a form inaccessible to extraction but released by digestive processes, but it also checks the possible occurrence of toxic derivatives of the insecticide, produced by plant chemistry, that may not be recorded by the method of analysis. Ideally, such parallel feeding tests should be of the long-period low-level type, since, to obtain an acute response, a degree of contamination may be needed for which the analysis might be more efficient than at the low levels at which it will be used in practice. The conclusion to be desired is that no physiological damage can be found after consumption for a sufficient period of vegetable product in which the analytical method can still detect insecticide. For reasons of practicability and economy of effort it may be necessary to build up this answer from several lines of evidence, such as parallel analytical and feeding trials at high level,[7,8] together with feeding trials in which toxicant is added quantitatively at both high and low levels. A large factor of safety is always to be included in any recommendations made by responsible authorities.

III. Clean-up procedure

The refinement necessary in cleaning up the extract from possibly interfering substances before the determination obviously depends on several factors, including the sensitivity and selectivity of the final procedure, the nature of crop or product and the lowest level of insecticide that must with certainty be detected. The necessity of distinguishing between insecticides, or between the original insecticide and some toxic derivatives thereof, may also influence the choice.

At present, no organo-phosphorus insecticide of strongly electrolytic nature is in use. Amiton exists mainly as a cation at the pH of plant tissues, but its ionisation is suppressed at higher pH values. Demeton-methyl can give rise to a fairly stable sulphonium salt, which is toxic by injection, but not particularly toxic by ingestion.[9] Products are formed by partial hydrolysis having a free acidic function on the phosphorus, but, once this stage has been reached, the molecule is no longer toxic.

All organo-phosphorus insecticides so far are, in non-ionic form, favourably partitioned into chloroform from water, owing to exceptionally strong molecular forces between $P \rightarrow O$ and $CHCl_3$ or to the corresponding $P \rightarrow S$ compounds having much reduced hydrophilic behaviour. The least favourably partitioned of the insecticides in use is schradan, which still partitions 7 to 1 in favour of chloroform. Derivatives produced in the plant, which are generally oxidation products, are more favourable to water, that from dimethoate for example partitioning about 1 to 1. More exhaustive extraction with chloroform is needed when these derivatives must be determined. Extraction from water into chloroform is therefore a favoured first step after aqueous maceration.

Partitioning from water into chloroform, however, does not by itself reduce interfering substances to a sufficiently low level. Various further steps have been used. Chromatography can be regarded as the "ultimate" procedure, but, in the interests of economy of time, it is always preferred to use some less complete separation if it is sufficient, such as further partitioning in a funnel, removal of easily absorbable substances on a short column, non-fractionating distillation or partial hydrolysis. Since the degree of cleaning up needed is necessarily influenced by the final determination step, these methods are dealt with in later sections.

If chromatography can be taken to the stage of complete isolation of the constituent to be determined, a non-specific method of final determination can be used. Gas - liquid chromatography with the thermal-conductivity method of detection has been explored as a possible tool for residue analysis.[10] As apparatus and experience in its use become more widespread, this method may find more extensive application, but, in our opinion, the non-specific nature of the final determination is strongly against it. Slight changes in volatility of natural compounds produced by differing substituents in chemically non-interfering compounds could upset seriously the conclusions, unless the method is thoroughly examined for each crop. With more specific detectors, once a means has been found of avoiding interference in a few types of crop, the method is likely to be general, needing only confirmation in others. The possibility of more specific methods of determining pesticides containing chlorine or sulphur is being explored.[11]

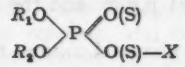
The object of the clean-up procedure is to reduce the contribution made to the final determination by natural plant products. It is not generally practicable to reduce this interference to zero; moreover, the interference, which is often due to unknown substances, may be extremely variable with the crop or even with its state of growth or subsequent history. It is therefore most important that blank determinations be made on samples of untreated crop, as similar as possible, except for treatment, to the crop treated with insecticide. The analyst should satisfy himself that the necessary comparison material is available and that the blank values, or at least the uncontrollable variations among them, are below the desired level of significance of the final determination. The analyst's task is frequently made more difficult and his results less certain by failure on the part of field workers, who may be many thousands of miles away, to appreciate the necessity to supply control material.

For determinations of cholinesterase the interference, as mentioned above, is more complex and in general greater than in more direct methods. It is therefore necessary to carry out parallel determinations on similar extracts of treated and untreated plant material with esterase and with insecticide added to the latter to provide the directly relevant calibration curve.

IV. Methods for the determination of organo-phosphorus insecticides

Most of the methods in use for determining residues are absorptiometric, since only colour reactions are generally sufficiently selective and sensitive. Volumetric or gravimetric methods are not (except with special elaboration) applicable to the microgram amounts to be determined nor sufficiently selective to be applied without an impracticable degree of separation of the insecticide from analytically interfering substances of plant origin.

The general structure of the organophosphate insecticides may be represented as—



where R_1 and R_2 are ethyl or methyl, occasionally isopropyl, and X is an organic radicle. The R_1 and R_2 substituent groups are less easily hydrolysed from the phosphorus than is the X group, and the derived alcohols are ubiquitous products of hydrolysis of natural substances. Their estimation offers no useful possibility for insecticide determination. In the phosphoramides, OR_1 and OR_2 are replaced by dimethylamino- or isopropylamino-groups. In one instance, dimethylamine liberated from a phosphoramide has formed the basis of a residue method. The analytical methods in the literature fall into three groups, depending on the part of the molecule used.

(1) Properties of the whole molecule.

(2) Determination of phosphorus.

(3) A reaction depending on some property of the group X, either while still attached to the phosphate radicle or after hydrolysis.

In considering reactions of the whole molecule the most important characteristic used for analytical purposes is the property of inhibiting the cholinesterase enzymes. This subject was briefly mentioned in the introduction; its further discussion would be outside the scope of this review. Many residue methods described utilise this principle, and a review of the procedures adopted is given by Schechter.[12]

Catalysis of the colour-producing reaction of hydrogen peroxide on certain aromatic bases by phosphoric anhydride compounds has been shown by Epstein, Rosenblatt and Demek[13] and Marsh and Neale[14] to provide a sensitive method for determining some of the phosphorus "nerve gases." How general this method is or whether it could be applied in the presence of plant extracts is not known.

The infra-red absorption spectrum is a highly characteristic property of any molecule. This method has been used for residues.[15] At present it is of limited application owing to marginal sensitivity and high demands on the clean-up technique. Hornstein[16] has reported on possible applications of fluorimetry and finds that guthion and Potasan are fluorescent in ultra-violet light. Polarographic procedures have been employed for residue determinations, but no specific references to organo-phosphorus compounds are available.

Methods depending on the determination of phosphorus or specific reactions of the rest of the molecule form the major methods of approach to the determination of residues. Each method has its own advantages and disadvantages; the choice between them can only be made with knowledge of the chemistry of each compound, and of the possible decomposition products that may occur in the plant, and must include consideration of the objective of the analysis. Phosphorus methods are non-specific, and the determination will include other phosphorus-containing insecticides unless these are removed in the clean-up procedure; non-phosphorus methods can often be made specific for a single insecticide, or at least for a group of related insecticides. Such specific methods are of advantage if one of several insecticides in a food crop needs to be determined. Specificity is not always desirable in a method to be applied to a compound that may be oxidised or metabolised on or in the plant. The total-phosphorus method in these circumstances may give a more accurate measurement of all toxic products derived from the insecticide in the crop.

Phosphorus methods are applicable to all compounds of this class and are capable of development into general non-specific methods for organo-phosphorus insecticides. When surface residues only are sought, solvent stripping and then hydrolysis and determination of phosphorus can be used.[17] For total residues or crops to which the previous simple technique cannot be applied, a clean-up from interfering phosphates is necessary. As already mentioned, chloroform extraction from water is not adequately selective. The selectivity is greatly improved by adding a distillation step. This was first applied to the most volatile of the organo-phosphorus insecticides,[5] dimefox, on a macro scale, without preliminary extraction, and later Otter[18] used micro-distillation of a chloroform extract under reduced pressure to isolate the residue from plant phosphates. This technique has been used for residues of schradan,[19] and dimethoate[7] and related compounds[20] and is probably more generally applicable. Recovery of added insecticide varies from 65 to 85 per cent. with different

crops, and an untreated sample is therefore desirable. The blank values on plants known to contain no insecticide are below 0·1 p.p.m. and the method has been applied to residues in all important food crops.

Laws and Webley[21] used a short charcoal column to absorb interfering substances from a chloroform solution before determining demeton-methyl residues in cabbage, lettuce and potatoes, Thimet (phorate) residues in peas and Phosdrin residues in kale and sugarbeet. Interference from plant phosphates was reduced to negligible proportions and recovery of added insecticide was 80 to 90 per cent. efficient. This technique is being investigated as a general method for organo-phosphorus insecticides.

Methods depending on the non-phosphorus group have been extensively developed. They all utilise a specific reaction of the substituent group and cannot readily be classified. In most of the methods reported the residue after suitable extraction and clean-up is hydrolysed with alkali to break the P–O(S)–C link, and then the liberated organic substituent is isolated and determined. Many examples of this type will be found under the compounds listed in section V.

With any method the recovery of the insecticide residue present in the sample is never complete and may vary considerably with different crops. It is essential that a method be tested on each crop by a recovery experiment in which a known amount of the insecticide to be determined is added to an untreated sample at an early stage in the analysis. Only recovery from an early stage of the laboratory procedure onwards can be measured in this way. The problem of efficiency of the initial extraction from the vegetable material must be dealt with by the methods discussed in section II.

The level of added insecticide in the "fortified" sample should be similar to the residue level expected in the sprayed crop. The isotope-dilution technique has been used in recovery experiments[22]; it has the advantage of application without appreciable change in the insecticide content of the sample, but it is still limited to the laboratory stages of the process. This application of radio labelling is distinct from its more valuable contribution, when applied to the growing crop, at an early stage of the investigation of methods for a new insecticide.

V. Methods for individual compounds

A summary of the methods available for the determination of residues of some insecticides is given below. The insecticides in current use in this country are included, together with some others for which methods are available. Previous reviews of the literature on determining insecticide residues, including organo-phosphorus compounds, will be found under references 23, 24, 25, 26, 27, 12 and 28.

Demeton—

A 65 + 35 mixture of demeton O (diethyl 2-ethylthioethyl phosphorothionate) and demeton S [diethyl *S*-(2-ethylthioethyl) phosphorothiolate]

Hensel *et al.*[29] used a method based on cholinesterase inhibition determined on an aqueous or chloroform extract of the crop. The cholinesterase depression observed was compared with those produced by standards prepared from demeton in an extract of the untreated plant. Metcalf *et al.*[30] compared the results of the anticholinesterase method with residues determined by ^{32}P-labelled demeton and concluded that the residues found by the enzymatic method would be high by a factor of 5 or 10 because of oxidation in the plant to more powerful inhibitors of cholinesterase. Van Middelem and Waites[31] report residues determined on collards, lettuce and mustard by the cholinesterase technique.

Demeton residues can be determined by the phosphorus methods described by Heath *et al.*[19]

Demeton-methyl—

A 70 + 30 mixture of demeton-O-methyl (2-ethylthioethyl dimethyl phosphorothionate) and demeton-S-methyl (*S*-2-ethylthioethyl dimethyl phosphorothiolate)

The chemical behaviour of demeton-methyl in the plant and the determination of residues with ^{32}P-labelled insecticide has been described by Tietz,[32] who exhaustively extracted the plant with methanol. After removing the solvent the residue is taken up in water and extracted first with light petroleum and finally with chloroform. The toxic residue is determined by measuring the radioactivity in the chloroform extract. This method is not applicable to residues in crops for human consumption. Laws and Webley[21] determined residues

by extraction of the crop with aqueous methanol or with isobutyl alcohol when a high blank value was encountered with methanol; plant interference was avoided by chromatography on a charcoal column in chloroform solution, and the insecticide was determined colorimetrically as phosphorus after wet combustion with nitric, hydrochloric and perchloric acids. The results agree with those determined by anticholinesterase measurements. Residues can also be determined by determination of phosphorus after purification by distillation.[23]

DIMEFOX—

NNN'N'-Tetramethylphosphorodiamidic fluoride

Only methods based on determining phosphorus are available for dimefox residues. Dupée, Heath and Otter[5] described a method of purification by distillation under reduced pressure from a glycerol - ethylene glycol solution that is applicable to brassica crops and strawberries, and co-distillation from a paraffin macerate of the crop used for cocoa beans and hops. Field and Laws[33] used chromatography on a magnesia column for dimefox residues in dried hops.

DIPTEREX (TRICHLORPHON)—

Dimethyl 2,2,2-trichloro-1-hydroxy ethylphosphonate

The anticholinesterase method proposed by Hensel *et al.*[29] has been used for residues of Dipterex on apples, French beans, lettuce and spinach.[23] A detailed study of the cholinesterase technique has been made[34] and corrections applied for errors arising from spontaneous hydrolysis of the substrate, and for the conversion of Dipterex to dichlorvos (2,2-bichlorovinyl dimethyl phosphate) during the determination. Residues between 0·2 and 1·5 p.p.m. can be determined by the modified procedure.

DIAZINON—

Diethyl 6-methyl-2-isopropyl-4-pyrimidinyl phosphorothionate

Suter *et al.*[35] determined diazinon by extracting the residue into 48 per cent. hydrobromic acid from a solution in light petroleum. The acid extract is boiled under reflux while a stream of nitrogen, which carries the hydrogen sulphide liberated into a zinc acetate absorbing solution, is passed through it. Methylene blue, which is determined at 670 mμ, is formed by reaction with *p*-aminodimethylaniline and ferric chloride. In Harris's method,[36] as modified by Blinn and Gunther,[37] diazinon is hydrolysed to 2-isopropyl-6-methyl-4-pyrimidol, interfering substances are removed by extraction from strongly acid and basic solutions and finally the pyrimidol is extracted into chloroform from a weakly acid solution and determined from its absorption at 272 mμ. Diazinon may also be determined by a phosphate method[17] by extracting the crop with light petroleum, washing the extract to remove water-soluble phosphates, hydrolysing the evaporated residue by heating under reflux with 48 per cent. hydrobromic acid and determining phosphorus by the molybdenum-blue reaction. These three methods have been compared[17] on the same samples and with results by a cholinesterase method and found to give excellent agreement above 1 p.p.m., but below this level there is some interference leading to divergent results. Read and Hughes[38] have investigated the methylene blue and the pyrimidol methods on mushrooms and found the former preferable in being less affected by interference from the mushroom extract.

DIMETHOATE—

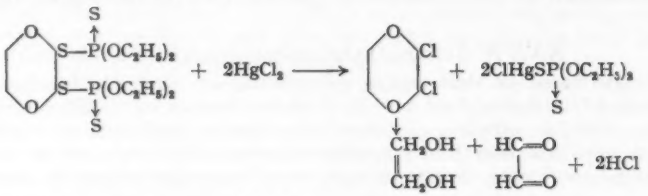
OO-Dimethyl *S*-(*N*-methylcarbamoylmethyl) phosphorothiolothionate

Residues on a wide variety of crops have been determined by micro-distillation and then determination of phosphorus.[7] The blank values on untreated crops are reduced to 0·1 p.p.m. and recoveries of 65 to 80 per cent. are obtained. Insecticide labelled with ^{32}P has been used to demonstrate the efficiency of the extraction technique, and feeding tests on animals indicate that the method determines the total residue hazard present in a sprayed crop. Santi and Bazzi[39] extracted cherries with a chloroform - acetonitrile mixture and liberated methylamine by hydrolysis with hydrochloric acid. Methylamine reacts with ninhydrin to produce formaldehyde, which is determined with chromotropic acid. The method will detect a residue of 0·2 p.p.m.; an alternative paper-chromatographic method will detect 0·6 p.p.m. of residue. Residues in olive oil have been determined by determining phosphorus after solvent extraction.[40] The phosphorus, methylamine and paper-chromatographic methods give recoveries of 70 to 80 per cent.

DELNAV—

The bis-(*OO*-diethyl phosphorothiolothionic) ester of 2,3-*p*-dioxandithiol

A specific method is described by Dunn[41] based on cleavage by mercuric chloride to glyoxal, which is converted as it is formed to the 2,4-dinitrophenylosazone, which gives an intense blue colour in alkaline solution.



The crop is minced and extracted with hexane, the extract is purified by adsorption on alumina containing 2 per cent. of water and extracting with benzene. Further purification is necessary by partition chromatography with acetonitrile on a Celite column. The cleavage reaction and osazone formation is carried out in one stage, the glyoxal 2,4-dinitrophenylosazone is isolated and purified by chromatography on alumina. The colour is developed by adding tetramethylammonium hydroxide to a solution in dimethylformamide. Gunther *et al.*[42] applied this method to residues on orange and lemon peel and reported recoveries of 106·5 and 86·0 per cent. of insecticide; they found the hexane extracts to be stable for up to 3 months when stored at 10° C.

GUSATHION (GUTHION)—

OO-Dimethyl *S*-(4-oxo-3*H*-1,2,3-benzotriazine-3-methyl) phosphorothiolothionate

Guthion can be hydrolysed[43] to a diazonium compound, which is coupled with phenyl α-naphthylamine to give a blue-violet colour; this test is specific for guthion, but has not been applied to residues. Hydrolysis to anthranilic acid and diazotisation and coupling with 1-naphthylethylenediamine has been employed for residue determinations.[44] Suitable clean-up procedures are described for cottonseed, fruit, milk and chlorophyll-containing crops, the final colour development being similar to Averell and Norris's method for parathion (*q.v.*). The sensitivity is 0·05 to 0·1 p.p.m. of guthion according to the nature of the sample, and studies on the corresponding oxygen analogue show that it is effectively recovered from green plants. Giang and Schechter[45] hydrolysed with hydrochloric acid; formaldehyde liberated from the methylene group was distilled and then determined with chromotropic acid reagent. The preliminary clean-up involves extraction of the residue with chloroform, separation by adsorption on alumina from a solution in pentane and subsequent elution with acetonitrile. Recoveries of 94 to 99 per cent. of guthion added to crushed cottonseeds are claimed and the oxygen analogue, *OO*-dimethyl *S*-(4-oxo-3*H*-1,2,3-benzotriazine-3-methyl) phosphorothiolate, is also determined by the method. Of other insecticides, Thimet, Trithion and Nialate [*SS*-bis-(*OO*-diethyl phosphorothioate)] liberate formaldehyde under these conditions. Archer and Zweig[46] determined the anticholinesterase activity of a purified extract, after oxidation, colorimetrically with indophenyl acetate as the chromogenic substrate.

MALATHION—

S-1,2-Di(ethoxycarbonyl)ethyl *OO*-dimethyl phosphorothiolothionate

Norris, Vail and Averell's method[47] forms the basis of all the residue methods described in the literature. The crop is extracted with carbon tetrachloride, the insecticide is decomposed, by alkali in ethanol - carbon tetrachloride solution, to sodium dimethyldithiophosphate and sodium fumarate. The former is extracted into water and converted to the copper complex, which is soluble in carbon tetrachloride and can be determined colorimetrically at 418 mμ. A collaborative examination of the method reported by Conroy[48] found it suitable for surface residues, and suggested possible improvements in technique. The basic procedure has been improved and modified for the determination of malathion in meat, fat, liver, milk and eggs.[49] By the methods described the blank values on samples known to contain no

malathion are reduced to below 0·2 p.p.m. and recovery values of 70 to 100 per cent. are quoted on all five products. A modified extraction for cottonseed is described by Norris and Kuchar.[50]

PARATHION—

OO-Diethyl *p*-nitrophenyl phosphorothionate

The most widely used method is that proposed by Averell and Norris,[51] in which the nitro-group is reduced to the amine, which is diazotised and coupled with *N*-1-naphthylethylenediamine. The adaptation of the method to the routine examination of a large number of samples was discussed by Gunther and Blinn.[52] The method of the Association of Official Agricultural Chemists (1955) is based on the same procedure. The sample is extracted with benzene, the extract is purified by shaking with a mixture of attaclay, kieselguhr and charcoal, and filtering. An aliquot of the clarified extract is reduced with zinc dust and hydrochloric acid, the filtered solution of amine is diazotised with sodium nitrite, and ammonium sulphamate and then *N*-1-naphthylethylenediamine hydrochloride solution are added. The optical density is measured at 550 mμ. A similar method was used by Gage[53,54] with *N*-β-sulphatoethyl-*m*-toluidine as the coupling agent. Bazzi and Santi[55] described the extraction of residues from olive oil by extraction with ether at −75° C. Vidic[56] modified Averell and Norris's procedure for blood and tissue. Buckley and Colthurst[57] determined parathion by alkali hydrolysis to *p*-nitrophenol, which was then determined at 408 mμ, interference from many plant pigments being overcome by oxidation with hydrogen peroxide during the hydrolysis.

PARATHION-METHYL, CHLORTHION AND EPN—

OO-Dimethyl *p*-nitrophenyl phosphorothionate
O-(3-Chloro-4-nitrophenyl) *OO*-dimethyl phosphorothionate
O-Ethyl *O*-*p*-nitrophenyl phenylphosphorothionate

For the analysis of these residues Averell and Norris's method is used. Kolbezen and Reynolds[58] used zinc in phosphoric acid for reducing Chlorthion residues in cottonseed to avoid yellow colours encountered in untreated samples with hydrochloric acid.

PHENKAPTON—

S-(2,5-Dichlorophenylthiomethyl) *OO*-diethyl phosphorothiolothionate

Hardon *et al.*[59] hydrolysed phenkapton to 2,5-dichlorothiophenol, which was then brominated to 2,5-dichlorophenylsulphonyl bromide. Reaction with potassium cyanide forms cyanogen bromide, which is determined colorimetrically with pyridine and benzidine by Aldridge's method. The method is relatively specific for phenkapton, the sensitivity claimed being about 5 μg, equivalent to 0·05 p.p.m. of residue in a 100-g sample. In a method based on condensation to a thioindigo-derivative,[60] the residue in methanol solution is condensed with chloroacetic acid in alkaline solution. The 2,5-dichlorophenylthioglycollic acid formed is purified and condensed in sulphuric acid to give a thioindigo-derivative, which is extracted into *o*-dichlorobenzene and determined at 560 mμ; 20 μg of phenkapton can be detected. Phenkapton can also be determined by the micro-distillation technique and by determining phosphorus after hydrolysis with hydrobromic acid, as used for diazinon.

PHOSDRIN—

1-Carbomethoxy-1-propen-2-yl dimethyl phosphate

Casida *et al.*[61] modified Hensel's cholinesterase method for the determination of Phosdrin residues down to a level of 0·05 p.p.m. Dormal *et al.*[62] used cholinesterase techniques for residues in spinach, peas and beans both as freshly harvested and after processing. Laws[63] has shown that Phosdrin may be determined by the chromatographic method for demeton-methyl. He compared the results of chromatographic and cholinesterase techniques and notes that the anticholinesterase method tends to give lower results in later-harvested samples, probably due to the quicker decomposition of the more active isomer.

PHOSPHAMIDON—

2-Chloro-2-diethylcarbamoyl-1-methylvinyl dimethyl phosphate

Phosphamidon residues have been determined as phosphorus after micro-distillation. Giang *et al.*[64] have used Hall, Stohlman and Schechter's method[65] for schradan as a basis for an analytical method for phosphamidon by hydrolysis to yield diethylamine.

SCHRADAN—

Bis-*NNN'N'*-tetramethylphosphorodiamidic anhydride

David *et al.*[66] extracted plant material by maceration with water and filtration and then hydrolysed natural plant phosphates by controlled alkaline hydrolysis, which does not affect schradan. Schradan is extracted by chloroform, digested with hydrochloric acid and perchloric acid and determined colorimetrically as phosphorus. Good recoveries are obtained, but some crops give a blank value of up to 1 p.p.m. Heath *et al.*[19] reduced plant interference to below 0·1 p.p.m. by vacuum distillation of the extracted residue. The plant is macerated with water, and the residue extracted into chloroform for alkaline solution. The dry chloroform extract is then distilled at 1 mm pressure and 100° C on to a cold-finger condenser. The condensate on the cold-finger condenser contains schradan residue freed from interfering plant extractives, and the schradan is determined colorimetrically as phosphorus after digestion with perchloric acid. One of the authors has used a temperature of 140° C in the distillation, which was found to give higher recoveries from some crops. Hall, Stohlman and Schechter[65] extracted the plant with chloroform, and hydrolysed in strong acid to liberate dimethylamine, which was steam-distilled from alkaline solution, absorbed in acid and determined as yellow cupric dimethyldithiocarbamate.

TRITHION—

S-(*p*-Chlorophenylthiomethyl) *OO*-diethyl phosphorothiolothionate

The extract after a chromatographic clean-up is hydrolysed by alkali in ethylene glycol to *p*-chlorothiophenol, which is determined colorimetrically with 2,6-dibromo-*N*-chloro-*p*-quinoneimide, the orange colour being measured at 480 mμ.[67] Gunther *et al.* used a total-chlorine method to determine residues on oranges and lemons.[68] Patchett[69] discussed the difficulties involved in determining Trithion by the cholinesterase method because of the presence of more actively anticholinesterase oxidation products in aged residues. The procedure described involves extraction, separation of oils and waxes with acetonitrile, oxidation in a hydrogen peroxide - acetic acid - benzene system and then a cholinesterase procedure for the quantitative determination. Giang, Adams and Schechter[70] have determined Trithion by hydrolysis with alkali after extraction and acidification to liberate formaldehyde, which is determined by colour reaction with chromotropic acid in a procedure similar to that used for Thimet residues.

THIMET (PHORATE)—

OO-Diethyl *S*-(ethylthiomethyl) phosphorothiolothionate

Bowman and Casida[71] have shown that Thimet and five oxidation products are possibly present in plant residues. Giang and Schechter[72] described a method for Thimet and all metabolites by oxidation of the extracted residue to the sulphone, hydrolysis with alkali and liberation of formaldehyde from the active methylene group by chromotropic acid and sulphuric acid. Recoveries of 85 to 95 per cent. of Thimet added to the crop are claimed. Residues in peas have been determined by a modification of Laws and Webley's method for demeton-methyl.[21]

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The Use of Selective Desorption from Carbon Columns for the Determination of Maltotriose in Starch Conversion Products

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In an earlier paper, the determination of dextrose and maltose in starch conversion products was described. The method has now been extended to the determination of maltotriose.

Full details are given in an earlier paper[1] for making and testing carbon columns and also for preparing and standardising the Somogyi reagent; these techniques were used in the work described here. It is therefore advisable to refer to the earlier paper, as only modifications of the original process are described here in detail.

The sugars present in a normal liquid glucose are shown in the chromatogram reproduced as Fig. 1 in the paper by Buchan and Savage.[2] It was thought that, by a simple extension of the method for separating dextrose and maltose on a carbon column, conditions could readily be found for determining the third fermentable sugar, maltotriose (sugar "*c*" on Buchan and Savage's chromatogram[2]), after removal of dextrose and maltose. However, the clean, quantitative separation of maltotriose from maltotetraose (sugar "*d*" on Buchan and Savage's chromatogram[2]) proved unexpectedly difficult.

With the type of column described[1] and with a batch of B.D.H. charcoal from which maltose could be readily eluted (batch No. 3 from the previous paper[1]), variations in temperature and concentration of ethanol were tried and histograms were plotted. The sugars present in fractions of the eluates were also identified qualitatively by paper chromatography. It was found that increasing the concentration of ethanol or increasing the temperature led to more rapid elution of maltotriose, as might be expected. Usually, it was followed by more rapid elution of some of the maltotetraose.

Different temperatures and different concentrations of ethanol gave varying success in the effective elution of maltotriose, but no completely satisfactory conditions were found for its separation from maltotetraose.

There was the possibility that the elution of small amounts of maltotetraose with the maltotriose might be the result of "overloading" of the column with respect to maltotetraose, but, in experiments in which half the usual amount of liquid glucose was put on to the column, interference by maltotetraose was not reduced.

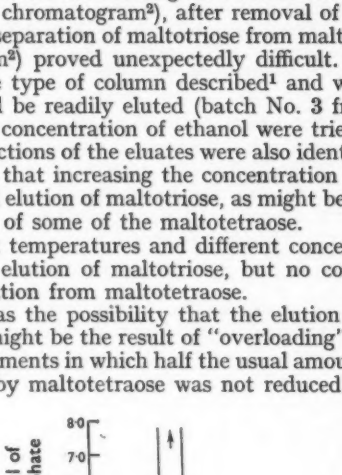

Fig. 1. Histogram for recovery of maltose from 6-inch × 0·75-inch column of batch No. 4 B.D.H. charcoal at 23° C

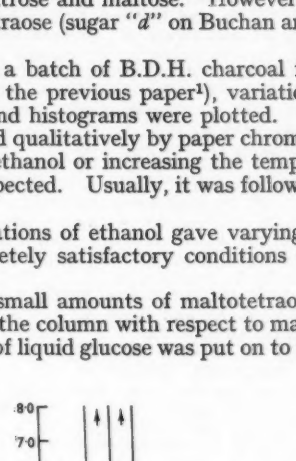

Fig. 2. Histogram for recovery of maltose from 15-inch × 0·5-inch column of batch No. 4 B.D.H. charcoal at 23° C

A longer and narrower column containing the usual amount of the carbon - kieselguhr mixture was found to give much sharper elution bands for the sugars, as, for example, is shown in Figs. 1 and 2, and it was decided to find out whether conditions existed whereby such a column could be used for the separation of maltotriose.

PROCEDURE FOR TESTING A BATCH OF CARBON WITH THE MODIFIED COLUMN—

It was realised that conditions of temperature and ethanol concentration for eluting maltose would not necessarily be the same for the longer column as they were for the shorter column, and the new conditions had to be determined before investigating the elution of maltotriose.

A column 15 inches long × 0·5 inch diameter was charged with 7 g of a mixture of equal parts by weight of carbon and kieselguhr, and, by pipette, 5 ml of a 2 per cent. solution of liquid glucose were placed on to it. The dextrose was then eluted with 100 ml of water. As a starting point, 7 per cent. ethanol was chosen for eluting the maltose, this being the concentration recommended for the shorter column, and the optimum temperature for the elution was determined. It was found that this temperature was a little lower than that required for the elution of maltose by this concentration of ethanol from the shorter column. It was now possible to investigate the elution of maltotriose from the long column after having removed dextrose and maltose; this investigation was carried out in the usual way by collecting 10-ml fractions of the eluate, determining the sugar in each fraction by the Somogyi copper-reduction method and checking the purity of the eluted sugar by evaporating the remainder of each fraction under reduced pressure and testing by paper chromatography. To determine whether or not any maltotriose had been retained by the carbon, the column was finally extracted with 30 ml of 50 per cent. ethanol. Half of this extract was then evaporated to small bulk, which was completely transferred to paper for chromatography. In this way, different concentrations of ethanol and temperatures could be tried. It was found that the best conditions for eluting maltotriose were a relatively low concentration of ethanol and a relatively high temperature. Thus for batch No. 3 B.D.H. charcoal it was found that the maltotriose was cleanly eluted by 150 ml of 8 per cent. ethanol at 40° C.

With this carbon the three lower sugars in liquid glucose could therefore be separated on the longer column, as described below.

(1) Elute with 100 ml of water at about 15° C—dextrose recovered.
(2) Elute with 100 ml of 7 per cent. ethanol at about 15° C—maltose recovered.
(3) Elute with 150 ml of 8 per cent. ethanol at 40° C—maltotriose recovered.

In practice it has been found more convenient to work throughout at one temperature for the elution of all three sugars. This temperature must of necessity be the high one required for the elution of the maltotriose, and the concentration of ethanol suitable for the elution of maltose at this higher temperature must therefore be determined. (Elution of dextrose is no problem as it is not adsorbed and can be eluted with water at any temperature.) For batch No. 3 B.D.H. charcoal it was found that at 40° C the maltose could be completely and cleanly eluted with 100 ml of 5 per cent. ethanol. The results of a separation of dextrose, maltose and maltotriose from liquid glucose on a 15-inch × 0·5-inch column charged with 7 g of a mixture of batch No. 3 B.D.H. charcoal and kieselguhr, the temperature being maintained at 40° C throughout, are shown in Fig. 3.

The purity of each faction shown in Fig. 3 was checked by paper chromatography, which confirmed that the three sugars had been separated cleanly. After the elution of these sugars the column was extracted with 50 per cent. ethanol, and paper chromatography of this solution showed that there had been no retention of dextrose, maltose or maltotriose.

To confirm that maltotriose was recovered quantitatively from the column under the conditions chosen, a known amount of this sugar was put on the column, elution was carried out in the usual way, and the sugar was determined in the eluate. Complete recovery was obtained.

PREPARATION OF PURE MALTOTRIOSE—

A sample of pure maltotriose was required to standardise the Somogyi reagent and also for testing the column to prove complete elution of this sugar. Maltotriose is not available commercially and it was necessary to prepare it in the laboratory. The source was ordinary liquid glucose and from this the maltotriose was separated by the carbon column as described above. The solutions of maltotriose obtained from two such columns were combined,

evaporated to small bulk under reduced pressure and applied as a streak across Whatman No. 3 chromatography paper. After development, the position of the maltotriose on the chromatogram was located by cutting strips from the paper and treating them with a suitable colour-forming reagent. That part of the paper containing the maltotriose was then cut

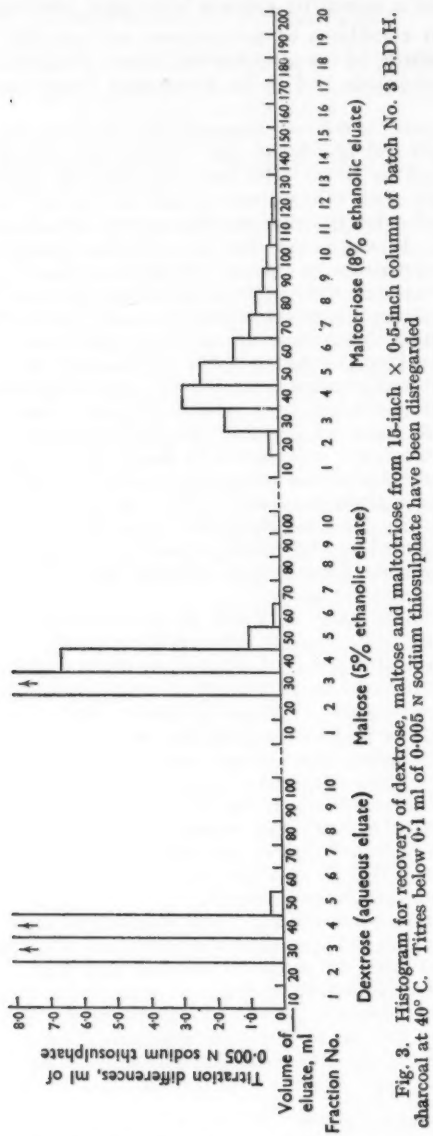
Titration differences, ml of 0·005 N sodium thiosulphate
Volume of eluate, ml
Fraction No.
Dextrose (aqueous eluate)
Maltose (5% ethanolic eluate)
Maltotriose (8% ethanolic eluate)

Fig. 3. Histogram for recovery of dextrose, maltose and maltotriose from 15-inch × 0·5-inch column of batch No. 3 B.D.H. charcoal at 40° C. Titres below 0·1 ml of 0·005 N sodium thiosulphate have been disregarded

out and eluted with a small amount of water. In this way a solution of some 20 mg of chromatographically pure maltotriose in about 3 ml of water was obtained. The density of this solution at 15·5° C was determined in a small pyknometer, and, by using the solution factor of 3·86, its sugar content was calculated. This method of determining sugar concentrations of this order when only a few millilitres of solution are available was tested with

a solution of maltose, and perfect agreement was obtained between the known concentration and the concentration found. The validity of the method depends on the assumption that a solution factor of 3·86 can be applied to maltotriose, but we consider that any error introduced by the use of this factor would be negligible.

A solution of maltotriose prepared in this way was suitably diluted and used to check recovery from a carbon column. It was also used to determine the heating time necessary for reaction of this sugar with the Somogyi reagent, the time found being 20 minutes, and to standardise the Somogyi reagent. Ideally, each batch of this reagent should be standardised, but in view of the difficulty of obtaining pure maltotriose the figures we obtained on a batch are shown below, and there would probably be little error if these were accepted for all batches made up in the same way.

Maltotriose (20 minutes' heating), mg ..	1·5	1·25	1·0	0·83	0·5	0·25
Volume of 0·005 N sodium thiosulphate, ml	3·73	3·03	2·43	1·97	1·20	0·54

MALTOTRIOSE CONTENT OF LIQUID GLUCOSE—

With batch No. 3 B.D.H. charcoal under the conditions shown in the histogram (Fig. 3), the dextrose, maltose and maltotriose contents for three different types of liquid glucose were determined. The dextrose and maltose contents have previously been reported.[3] After removal of the three sugars, the column was washed with 30 ml of 50 per cent. ethanol, half of it was evaporated to near-dryness for chromatography, and the absence of maltotriose was confirmed. A portion of the maltotriose eluate was similarly treated, and the presence of pure maltotriose was confirmed. Results for the determination of dextrose, maltose and maltotriose in liquid glucose are shown in Table I.

TABLE I

DEXTROSE, MALTOSE AND MALTOTRIOSE CONTENTS OF LIQUID GLUCOSE

Manufacturer's description of sample	Dextrose found, % w/w	Maltose found, % w/w	Maltotriose found, % w/w
Normal (43° Baumé)	16·4	11·3	11·1
Low dextrose equivalent (41·5° Baumé)	10·3	8·2	8·7
High dextrose equivalent (43° Baumé) ..	22·5	13·2	11·1

We have found that use of the longer column described above is more time-consuming and in general less convenient than the original shorter column. We would therefore recommend that, where figures for only dextrose and maltose are required, the short column should be used as originally proposed, and the longer column should be used only when a figure for maltotriose is also required.

We thank the Government Chemist, Department of Scientific and Industrial Research, for permission to publish this paper.

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An Examination of the Occurence of Honeydew in Honey

Part II*

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Twenty-eight honeys were analysed for moisture, ash, colloid, dextrin and reducing sugars, and values for specific rotation and pH were measured. The efficiency of Kirkwood, Mitchell and Smith's linear discriminant function, based on the results for ash, pH and reducing sugars, for distinguishing honeydew from floral honey (in particular ling-heather honey) was confirmed. Eleven of the samples tested contained honeydew.

Plants normally produce sufficient nectar to supply the bee colony with enough honey to ensure its survival, but in periods of prolonged drought the flow of nectar greatly diminishes, and the bees must augment the supply of honey from other sources of sugar. In such conditions, they will often collect honeydew, a sweet and sticky fluid excreted on foliage by leaf-sucking insects, mainly aphids and scale insects. The collection of honeydew is a nuisance to beekeepers, since the honey then produced is often too dark and rank-flavoured for sale and, more important, bees feeding on it may be greatly weakened or killed. As the obvious method of preventing harm to the bees is to remove honeydew honey from the hive, its identification is important.

In a previous paper,[1] Kirkwood, Mitchell and Smith reported the analysis of forty-two honeys for moisture, colloid, nitrogen, dextrin, reducing sugars, free acidity and ash; specific rotation and pH were also measured. The results were subjected to discriminatory analysis, and a linear discriminant function, X, was evolved, the value of X being given by the equation—

$$X = -8{\cdot}3x_1 - 12{\cdot}3x_2 + 1{\cdot}4x_3$$

in which x_1 is the pH, x_2 is the percentage of ash and x_3 is the percentage of reducing sugars. The numerical value of this function for any sample serves to classify the sample as floral or honeydew in origin. The mean values of X for authentic floral and honeydew honeys were found to be 86·7 and 57·6, respectively. In practice, any honey found to have a value of X greater than 73·1 may be classified as floral, and a honey having a value less than 73·1 may be classified as honeydew.

Scottish heather honey is often very dark in colour and may resemble honeydew honey in appearance. Various workers[2,3,4] have analysed eighty-eight samples of heather honey, mainly ling, many of which gave high values for ash, pH and colloid content. However, the reducing-sugar contents of these samples were not determined, so that the value of the discriminant function[1] could not be calculated.

The object of this work was to test the efficiency of the discriminant function in distinguishing honeydew from floral honey, in particular Scottish heather honey, and, if necessary, to evolve a supplementary discriminant function for distinguishing honeydew from ling-heather honey, the higher colloid content of the latter being included as an extra term in the original function.

Methods

Ash, colloid, pH and moisture—

The cleaning of samples and determinations of sulphated ash, colloid, pH and moisture content were carried out as described previously.[3]

Reducing sugars—

Lane and Eynon's volumetric method,[5] involving reduction of Fehling's solution and the use of methylene blue as internal indicator, was used.

* For details of Part I of this series, see reference list, p. 165.

TABLE I

VALUES FOUND FOR VAR

Sample No.	Type of honey*	Locality	Suggested source	Approximate date of extraction	Colour	State
18G	F	Perthshire, Scotland	Raspberry and clover	1951	Lemon - buff	Fine granulation
16G	F	Ballindarroch, Scotland	Bell heather	1954	Dark brown	Coarse granulation
17G	F	Drummond Hill, Scotland	Lime and clover	1955	Lemon	Fine granulation
15G	F	Alness, Scotland	—	1944	Dark brown	Coarse granulation
13G	F	Oxford, England	—	1951	Dark brown	Very coarse granulation
25G	F	Angus and Dinnet, Scotland	Raspberry and clover	1959	Yellow - brown	Beginning to granulate
23G	F	Kincardineshire, Scotland	Ling heather	1952	Red - brown	Coarse granulation
9G	H	Grafrath, Germany	Pine (*Picea excelsa*)	1951	Light brown	Beginning to granulate
21G	F	Loch Lomond area, Scotland	White clover	1951	Dark brown	Coarse granulation
4G	H	Switzerland	Forest (honeydew)	1952	Green - brown	Liquid
1G	H	Puchberg, nr. Vienna, Austria	Common spruce and silver fir	1959	Dark amber	Liquid
2G	H	Lunz, nr. Vienna, Austria	Common spruce and raspberry	1959	Dark amber	Liquid
24G	F	Loch Davon, Aberdeen, Scotland	Ling heather	1959	Apricot	Liquid
6G	H	Deggendorf, Germany	Mainly pine, some fir and beech	1959	Brown - khaki	Beginning to granulate
22G	F	Loch Kinord, Aberdeen, Scotland	Ling heather	1959	Dark buff	Fine granulation
11G	H	Scheibbs, Austria	Pine (*Picea excelsa*)	1959	Buff	Fine granulation
14G	F	Muir of Ord, Ross-shire, Scotland	Pressed heather	1956	Apricot	Fine granulation
26G	F	Lumphanan, Aberdeen, Scotland	Blaeberry (*Vaccinium myrtillus*)	1959	Peach	Liquid
10G	H	Scheibbs, Austria	Fir (*Abies pectinata*)	1956	Dark green	Very viscous liquid
28G	F	Ballindarroch, Scotland	Ling heather	1959	Buff	Very fine granulation
7G	H	Landshut and Passau, Germany	Meadowland and pine-wood	1959	Brown - orange	Liquid
3G	H	Germany	—	1951	Dark brown	Very coarse granulation
5G	H	Deggendorf, Germany	Mainly pine, some fir and beech	1959	Dark khaki	Very viscous liquid
8G	H	Frieberg, Germany	Fir (*Abies pectinata*)	1959	Khaki - green	Liquid
19G	F	Kyle of Lochalsh, Scotland	Bell heather	1950	Brown - orange	Medium granulation
27G	F	Ballindarroch, Scotland	Mainly ling	1959	Buff	Very fine granulation
12G	F	Ballindarroch, Scotland	Ling heather	1958	Apricot	Very fine granulation
20G	F	Kyle of Lochalsh, Scotland	Pressed heather	1959	Buff	Fine granulation

* Floral honey = F; honey

Table I

Values found for various honeys

roximate late of traction	Colour	State	Flavour
1951	Lemon - buff	Fine granulation	Very sweet
1954	Dark brown	Coarse granulation	Syrupy
1955	Lemon	Fine granulation	Very sweet
1944	Dark brown	Coarse granulation	Treacle
1951	Dark brown	Very coarse granulation	Treacle
1959	Yellow - brown	Beginning to granulate	Very sweet
1952	Red - brown	Coarse granulation	Sweet
1951	Light brown	Beginning to granulate	Caramel
1951	Dark brown	Coarse granulation	Treacle
1952	Green - brown	Liquid	Fairly sweet
1959	Dark amber	Liquid	Very sweet
1959	Dark amber	Liquid	Very sweet
1959	Apricot	Liquid	Fruity
1959	Brown - khaki	Beginning to granulate	Very sweet
1959	Dark buff	Fine granulation	Very sweet
1959	Buff	Fine granulation	Fairly sweet
1956	Apricot	Fine granulation	Clover
1959	Peach	Liquid	Fruity
1956	Dark green	Very viscous liquid	Treacle
1959	Buff	Very fine granulation	Fairly sweet
1959	Brown - orange	Liquid	Very sweet
1951	Dark brown	Very coarse granulation	Rum
1959	Dark khaki	Very viscous liquid	Fairly sweet
1959	Khaki - green	Liquid	Very sweet
1950	Brown - orange	Medium granulation	Fairly sweet
1959	Buff	Very fine granulation	Fairly sweet
1958	Apricot	Very fine granulation	Fairly sweet
1959	Buff	Fine granulation	Strong (sweet)

* Floral honey = F; honeydew honey = H

HONEYS

lavour	pH	Ash on dry material, %	Dextrin on dry material, %	Specific rotation, α_D^{20} degrees	Colloid on dry material, %	Moisture content, %	Reducing sugars on dry material, %	Value of discriminant function (X)
weet	4·00	0·76	1·41	−12·82	0·17	19·2	92·6	87·0
y	4·17	0·65	3·04	−5·23	0·72	18·6	84·0	75·0
weet	4·24	0·16	1·00	−11·73	0·12	17·0	94·3	94·8
e	4·30	0·70	3·00	−3·21	0·34	19·9	85·2	74·9
e	4·37	0·56	0·44	−22·80	1·75	21·0	88·8	81·1
weet	4·40	0·18	1·61	−14·10	0·43	16·6	93·4	92·0
	4·42	0·43	1·02	−16·10	1·13	14·2	93·8	89·3
el	4·48	1·18	3·49	+12·48	0·11	13·5	75·2	53·6
e	4·48	0·57	3·45	−1·97	0·26	18·2	86·2	76·5
sweet	4·58	0·91	3·13	+9·54	0·31	16·0	74·8	55·5
sweet	4·70	0·90	4·16	+4·90	0·39	16·1	77·3	58·1
sweet	4·70	0·99	4·81	+4·29	0·36	17·2	81·7	63·2
y	4·70	0·49	1·20	−21·72	1·49	18·5	96·3	89·8
sweet	4·71	0·91	4·04	+4·11	0·38	15·1	78·0	58·8
sweet	4·73	0·49	0·38	−18·13	1·71	18·5	96·8	90·2
sweet	4·74	0·85	3·57	+3·48	0·24	17·4	76·9	57·8
r	4·74	0·64	1·14	−14·32	2·18	20·1	91·9	81·3
y	4·76	0·85	2·15	−10·47	0·29	18·1	88·2	73·5
le	4·78	1·14	2·98	+9·55	0·31	16·1	75·5	52·0
sweet	4·82	0·50	1·24	−17·40	1·67	15·9	91·2	81·5
sweet	4·83	0·88	9·76	+11·27	0·30	16·4	67·6	43·7
	4·89	1·31	5·37	+9·35	0·13	19·4	72·3	44·5
sweet	4·92	0·95	5·85	+4·94	0·27	13·9	77·9	55·5
sweet	4·96	1·01	4·45	+2·87	0·24	14·2	76·7	53·8
y sweet	4·99	0·31	0·62	−18·30	0·64	19·2	95·9	89·1
y sweet	5·05	0·44	0·83	−19·20	1·65	18·3	91·9	81·3
y sweet	5·11	0·64	0·83	−17·69	2·28	19·9	94·2	81·6
g (sweet)	5·42	0·56	0·40	−19·46	2·00	19·6	96·3	82·9

honey = H.

SPECIFIC ROTATION AND DEXTRIN CONTENT—

The determinations of specific rotation and dextrin content (by precipitation with ethanol) were carried out as described previously.[1]

RESULTS

The results of the various tests, arranged in order of increasing pH, are shown in Table I; Table II shows the ranges of values obtained in some of the tests.

TABLE II

RANGES OF VALUES FOR FLORAL AND HONEYDEW HONEYS

	Floral honey			Honeydew honey		
	Minimum	Maximum	Average	Minimum	Maximum	Average
Colloid content, %	0·12	2·28	1·11	0·11	0·39	0·28
Dextrin content, %	0·38	3·45	1·40	2·98	9·76	4·69
Specific rotation, degrees ..	−22·80	−1·97	−14·39	+2·87	+12·48	+6·98
Reducing-sugar content, % ..	84·0	96·9	91·8	67·7	81·7	75·8
Ash content, %	0·16	0·85	0·53	0·85	1·31	1·00
pH	4·00	5·42	4·63	4·48	4·96	4·75
Discriminant function, X ..	73·5	94·8	83·6	43·7	63·2	54·3

Ling-heather honey was readily identified by its high colloid content. As before,[1] for samples having the same colloid content, honeydew honeys were much darker, possibly indicating that less of their colouring matter was present in a colloidal state.

The dextrin contents found for honeydew honeys, greater than those of the floral honeys, were in agreement with the previous results.[1]

All the honeydew honeys were dextro-rotatory and all the floral honeys laevo-rotatory.

The reducing-sugar contents of the honeydew honeys were markedly lower than those of the floral honeys; this agrees with previous results.[1]

The ash contents of the honeydew honeys were slightly higher than those of the ling-heather honeys, but the ranges of values found for both types were not sufficiently different to give a reliable test for honeydew honey.

Measurements of pH were found to be useless in discriminating between ling-heather and honeydew honeys, as the ranges of pH values for both types of honey were almost coincident.

Values of the discriminant function, X,[1] were calculated and found to give satisfactory discrimination between the honeydew and floral honeys. The average values of X for honeydew and floral honeys agreed closely with those found previously[1] and with more recent unpublished results (September, 1960; personal communication from J. W. White, jun., U.S. Department of Agriculture, Agricultural Research Service, Philadelphia, Pa.). White, in an investigation of five hundred American honeys, obtained average values for X of 88·3 for floral honey and 56·4 for honeydew honey.

We gratefully acknowledge the valuable help given by many beekeepers, who supplied the samples of honey, and in particular by Dr. E. P. Jeffree, Aberdeen University, who arranged for the collection of samples. We also thank Mr. A. Cochrane for practical assistance.

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NOTE—Reference 1 is to Part I of this series.

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The Direct Absorptiometric Determination of Copper in Steel and Cast Iron with 2,2′-Biquinolyl

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Hitherto, the absorptiometric determination of copper in steel and cast iron by means of 2,2′-biquinolyl has involved a liquid - liquid extraction whereby cuprous ions are transferred to an organic liquid containing the reagent. Such an extraction is often undesirable in routine work, and a method has been developed whereby the copper - 2,2′-biquinolyl complex is formed in an aqueous solution containing *NN*-dimethylformamide. The method is eminently suitable for rapid routine work, and its over-all accuracy is shown by results for plain-carbon and alloy steels and cast irons.

Several organic reagents have been used for the absorptiometric determination of copper in steels and irons. These include compounds such as sodium diethyldithiocarbamate,[1] biscyclohexanone oxalyldihydrazone[2,3] and 2,2′-biquinolyl (cuproin)[4,5,6,7]; the last-named reagent is of particular interest by virtue of its specificity for the cuprous ion and formed the basis of this investigation.

The methods so far described for determining copper in steels by means of 2,2′-biquinolyl involve extraction of the cuprous ion from an aqueous solution into a solution of the reagent in isopentyl alcohol under conditions of controlled pH. The copper content is then assessed by absorptiometric measurement of its magenta-coloured complex. Although this procedure yields accurate results for a wide range of steels, it involves a liquid - liquid extraction, which is undesirable in a laboratory where many simultaneous determinations are often required. This paper describes the development of a procedure not including liquid - liquid extraction.

Experimental

Pflaum, Popov and Goodspeed[8] have shown that the copper - 2,2′-biquinolyl complex can be successfully produced in *NN*-dimethylformamide at pH 5 to 6, and, as dimethylformamide is miscible with water, its use was considered a promising beginning to the development of a method not involving a liquid - liquid extraction. The preliminary work was carried out with solutions containing known amounts of iron and copper to simulate the conditions of an acid solution of a plain-carbon steel. Each of five 0·5000-g samples of high purity iron (British Chemical Standard No. 149/1) was dissolved in 5 ml of hydrochloric acid, sp.gr. 1·16 to 1·18, and the solution was oxidised by the dropwise addition of 1 to 2 ml of nitric acid, sp.gr. 1·42. To the five solutions were added 0, 2, 4, 8 or 12 ml of a standard copper solution (1 ml ≡ 0·1 mg of copper), and each was then evaporated to dryness. The residues were moistened with 1-ml portions of the hydrochloric acid, again evaporated to dryness and baked for 1 to 2 minutes; they were then extracted with 2-ml portions of the hydrochloric acid, and the five solutions were diluted with water to 100 ml in calibrated flasks.

Use of hydroxylamine hydrochloride—

Initial experiments involved the use of hydroxylamine hydrochloride and tartaric acid, as these reagents were satisfactory in the liquid - liquid extraction method for copper.

To a 10-ml aliquot of each prepared solution were added 1 g of tartaric acid and 5 ml of hydroxylamine hydrochloride solution (15 per cent. w/v), and iron and copper were reduced by heating the solutions near their boiling-points until colourless. When the solutions were cool, the pH of each was adjusted to between 5 and 7 by the dropwise addition of sodium hydroxide solution (30 per cent. w/v). The solutions became yellow in colour, and the addition to each of 10 ml of 2,2′-biquinolyl solution (0·06 per cent. w/v in *NN*-dimethylformamide) resulted in almost immediate precipitation. Further experiments in which the acidity was adjusted before the hydroxylamine hydrochloride solution was added resulted in incomplete reduction and also produced precipitation after the 2,2′-biquinolyl solution had been added.

The effects of omitting the adjustment of acidity were next investigated. A series of 10-ml aliquots of the prepared solutions were treated as described above, but without the

addition of sodium hydroxide solution, *i.e.*, the 2,2′-biquinolyl solution was added immediately after the reduction and cooling stages. Clear magenta-coloured solutions were produced, and each was diluted to 50 ml in a calibrated flask. The optical densities of these solutions were measured against water in 4-cm cells with a Spekker absorptiometer, a mercury-vapour lamp, Calorex H503 heat-resisting filters and Wratten No. 74 light filters being used; the results were—

Copper present, mg	0	0·2	0·4	0·8	1·2
Optical density	0·051	0·170	0·304	0·560	0·816

Although these results showed promise, in that the calibration graph plotted from them was approximately linear, the absence of an acidity-adjustment stage made it essential to study the influence of acid concentration. Three 10-ml aliquots were taken from each of two of the prepared solutions, and 0-, 2- and 5-ml portions of 5 per cent. v/v hydrochloric acid were added to each group. The solutions were treated with tartaric acid, etc., as before, each was diluted to 50 ml after formation of the copper complex, and their optical densities were measured. The results, which clearly show that acidity has a pronounced influence on the calibration graph, were—

Hydrochloric acid (5 per cent.) added, ml	0	2	5
Optical density (4-cm cells) for 0·4 mg of copper	0·304	0·255	0·200
Optical density (4-cm cells) for 1·2 mg of copper	0·816	0·740	0·682

Several attempts were made to control the acidity with ammonium acetate and tartrate buffers, but all resulted in precipitation after the 2,2′-biquinolyl solution had been added.

TABLE I

EFFECTS OF AMOUNTS OF REAGENTS ON OPTICAL DENSITY OF SOLUTION CONTAINING 0·8 mg OF COPPER

Optical densities were measured against water in 4-cm cells

Tartaric acid added, g	Hydroxylamine hydrochloride solution (15 per cent. w/v) added, ml	2,2′-Biquinolyl solution (0·06 per cent. w/v) added, ml	Optical density in absence of hydrochloric acid	Optical density in presence of 5 ml of 5 per cent. hydrochloric acid
1	5	5	0·285	—
		10	0·540	—
2	5	10	0·561	—
4	5	10	0·548	—
1	10	10	0·541	0·430
1	5	15	0·618	0·520
		20	0·648	0·576
		25	0·679	0·676
Nil	5	25	0·660	0·658
		25	0·668	0·670

At this stage it was decided not to pursue the control of acidity, but to study the effects of varying the amounts of reagents used. Several 10-ml aliquots of one of the prepared solutions were treated as already described, but with different amounts of hydrochloric acid, hydroxylamine hydrochloride and 2,2′-biquinolyl solutions. The amounts of reagents used and the optical densities of the final solutions (after adjustment of their volumes to 50 ml) are shown in Table I. These results indicate that the use of tartaric acid is apparently superfluous and that, when the volume of 2,2′-biquinolyl solution is increased to 25 ml, precise control of acidity is unnecessary. An attempt was then made to produce a calibration graph. Aliquots (10 ml) of freshly prepared solutions containing known amounts of iron and copper were reduced by warming with 5 ml of the hydroxylamine hydrochloride solution; when cool, 25 ml of the 2,2′-biquinolyl solution were added to each, and the volumes of the solutions were adjusted to 50 ml in calibrated flasks. The optical densities were measured against water as before; the results were—

Copper present, mg ..	0	0·2	0·4	0·6	0·8	1·0
Optical density (4-cm cells)	0·095	0·235	0·386	0·532	0·688	0·832

Although the calibration graph plotted from these results was approximately linear, it could only be reproduced when the optical densities were measured immediately after final

dilution. Subsequent colour-stability tests (see Table II) showed that the optical density of a solution increased with time of standing; the same effect was noticed for a prepared solution containing no copper, the deepening brown colour presumably arising from re-oxidation of iron.

TABLE II

STABILITY OF COLOUR IN SOLUTIONS REDUCED WITH HYDROXYLAMINE HYDROCHLORIDE

Each solution was reduced with 5 ml of hydroxylamine hydrochloride solution (15 per cent. w/v) and colour was developed with 25 ml of 2,2′-biquinolyl solution (0·06 per cent. w/v). Optical densities were measured against water in 4-cm cells

Standing time, minutes	Optical density of solution containing— 0·6 mg of copper	1·0 mg of copper
0	0·532	0·832
5	0·531	—
10	0·535	—
15	0·538	—
20	0·541	—
25	0·548	—
30	0·553	0·855
90	0·598	0·903

ALTERNATIVE REDUCING AGENTS—

The instability of the colour appeared to arise, at least in part, from re-oxidation, and consideration was given to other reducing agents; of these, sulphurous and ascorbic acids seemed likely possibilities. Preliminary tests suggested that ascorbic acid was preferable, as its solution was easier to handle and, further, it reduced ferric ions in the cold. Aliquots (10 ml) of two of the prepared solutions were treated with 5 ml of ascorbic acid solution (20 per cent. w/v), which resulted in their immediate discoloration. After the addition of 25 ml of the 2,2′-biquinolyl solution, the volume of each solution was adjusted to 50 ml in a calibrated flask, and the optical densities of the solutions were measured at 10-minute intervals. The results (see Table III) showed that colour stability had been achieved.

TABLE III

STABILITY OF COLOUR IN SOLUTIONS REDUCED WITH ASCORBIC ACID

Each solution was reduced with 5 ml of ascorbic acid solution (20 per cent. w/v) and colour was developed with 25 ml of 2,2′-biquinolyl solution (0·06 per cent. w/v). Optical densities were measured against water in 4-cm cells

Standing time, minutes	Optical density of solution containing— 0·6 mg of copper	1·0 mg of copper
0	0·475	0·776
10	0·470	—
20	0·471	—
30	0·470	0·768
40	0·471	—
50	0·471	—
60	0·471	0·774

Attention was then turned to the influence of the concentration of acid on optical density. Aliquots (10 ml) from two of the prepared solutions were treated as described above with ascorbic acid and 2,2′-biquinolyl solutions. Different amounts of 5 per cent. v/v hydrochloric acid were initially added, and, although a 5-ml portion of ascorbic acid solution was used throughout, both the volume and concentration of the 2,2′-biquinolyl solution were varied. The results are shown in Table IV and indicate that the use of 25 ml of a 0·06 per cent. w/v solution of 2,2′-biquinolyl in *NN*-dimethylformamide produces solutions in which

optical density is not significantly influenced by the concentration of acid. It is of interest to note that increased stability of the colour to acid results from an increase in the amount of 2,2′-biquinolyl as well as in the volume of *NN*-dimethylformamide present.

TABLE IV

EFFECT OF VARIATION IN VOLUME AND CONCENTRATION OF 2,2′-BIQUINOLYL SOLUTION ON SENSITIVITY OF SYSTEM TO CONCENTRATION OF HYDROCHLORIC ACID

Each solution was reduced with 5 ml of ascorbic acid solution (20 per cent. w/v). Optical densities were measured against water in 4-cm cells

Copper present, mg	Volume of 5 per cent. v/v hydrochloric acid added, ml	Optical density of colour produced by— 10 ml of 0·06 per cent. w/v 2,2′-biquinolyl solution	25 ml of 0·06 per cent. w/v 2,2′-biquinolyl solution	10 ml of 0·15 per cent. w/v 2,2′-biquinolyl solution	10 ml of 0·024 per cent. w/v 2,2′-biquinolyl solution
0·6	0	0·460	0·472	0·468	0·458
	2	0·429	0·468	0·460	0·452
	5	0·417	0·467	0·454	0·444
1·0	0	0·753	0·780	0·765	0·758
	2	0·732	0·778	0·745	0·750
	5	0·704	0·775	0·732	0·718

As a final test of these conditions, calibration results were obtained from 10-ml portions of freshly prepared solutions containing known amounts of copper and iron. Each portion was treated with 5 ml of ascorbic acid solution (20 per cent. w/v) and 25 ml of 2,2′-biquinolyl solution (0·06 per cent. w/v). The volume of each solution was adjusted to 50 ml in a calibrated flask, and optical-density measurements were made against water in 4-cm cells; the results, which were reproducible, were—

Copper present, mg	0	0·2	0·4	0·6	0·8	1·0
Optical density	0·042	0·183	0·327	0·458	0·616	0·756

APPLICATION TO SAMPLES OF STEEL—

Although promising results were obtained when the procedure outlined above was applied to British Chemical Standard samples of mild steels, solutions of complex steels gave large positive errors, owing to the presence of chromium, etc. This difficulty was satisfactorily overcome by measuring optical densities against a compensating solution instead of against water; a suitable solution was prepared by adding 5 ml of the ascorbic acid solution and 25 ml of *NN*-dimethylformamide to 10 ml of the steel solution. This procedure also compensated for any coloured complexes that might be formed with the ascorbic acid, *e.g.*, the yellow titanium - ascorbic acid complex. Typical calibration results when optical densities were measured against the compensating solution were—

Copper present, mg	0	0·2	0·4	0·6	0·8	1·0
Optical density measured	0·013	0·168	0·310	0·465	0·606	0·755
Optical density corrected for blank	—	0·155	0·297	0·452	0·593	0·742

METHOD

APPARATUS—

Absorptiometer—A suitable instrument for optical-density measurement is a Spekker absorptiometer fitted with a mercury-vapour lamp, Calorex H503 heat-resisting filters and Wratten No. 74 light filters. (It was confirmed that the absorption spectrum of the copper - 2,2′-biquinolyl complex has a maximum at 545 mμ, and Ilford No. 604 or Wratten No. 74 light filters were therefore suitable; the Wratten filters were preferred, as they produce better instrumental response.) For samples containing up to 0·25 per cent. of copper, measure optical densities in 4-cm cells.

REAGENTS—

Hydrochloric acid, sp.gr. 1·16 *to* 1·18.

Nitric acid, sp.gr. 1·42.

Ascorbic acid solution, 20 per cent. w/v—Dissolve 20 g of L-ascorbic acid in water, and dilute to 100 ml. Prepare this solution freshly each day.

2,2′-Biquinolyl solution, 0·06 per cent. w/v—Dissolve 0·15 g of 2,2′-biquinolyl in 250 ml of *NN*-dimethylformamide, and store the solution in a dark bottle.

Standard copper solution—Dissolve 1·0000 g of analytical-reagent grade copper in the minimum volume of nitric acid, sp.gr. 1·42, boil gently to remove nitrous fumes, cool, and dilute to 1 litre in a calibrated flask. Transfer 100 ml of this solution to a 1-litre calibrated flask, and dilute to the mark with water.

1 ml ≡ 0·1 mg of copper.

PROCEDURE—

Weigh 0·5000 g of sample (see Note 1) into a squat 100-ml beaker, and add 5 ml of hydrochloric acid. When solution is almost complete, oxidise by adding 1 to 2 ml of nitric acid dropwise, and evaporate the solution to dryness. Cool, moisten the residue with 1 ml of hydrochloric acid, and again evaporate to dryness. Bake the residue for approximately 2 minutes, cool, add 2 ml of hydrochloric acid, and warm to dissolve soluble salts. Add 50 ml of water (see Note 2), cool, and transfer to a 100-ml calibrated flask. Dilute exactly to the mark with water, transfer two 10-ml aliquots to separate 100-ml beakers, and continue as described below.

To one aliquot (the test solution) add 5 ml of ascorbic acid solution, and mix thoroughly. Add 25 ml of 2,2′-biquinolyl solution, and mix again. Transfer to a 50-ml calibrated flask, dilute exactly to the mark at 20° C (see Note 3), mix, and set aside for 5 minutes. To the second aliquot (the compensating solution) add 5 ml of ascorbic acid solution, and mix. Add 25 ml of *NN*-dimethylformamide, mix, and transfer to a 50-ml calibrated flask. Dilute exactly to the mark at 20° C (see Note 3), mix, and set aside for 5 minutes.

Measure the optical density of the test solution against the compensating solution in 4-cm cells (see Note 1), deduct the blank value obtained by carrying out the procedure with a sample of pure iron (see Note 4), and obtain the copper content by reference to a calibration graph.

Preparation of calibration graph—Weigh a 0·5000-g sample of pure iron, *e.g.*, British Chemical Standard No. 149/1, into each of seven beakers, add 5 ml of hydrochloric acid to each, and oxidise by adding nitric acid dropwise. To the solutions add 0, 2, 4, 6, 8, 10 or 12 ml of standard copper solution, evaporate to dryness, and treat each residue as described above. Measure the optical density of each standard test solution against its compensating solution, deduct from each value the optical density of the solution containing no copper (see Note 4), and plot a graph relating optical density to weight of copper present.

NOTES—

1. For copper contents up to 0·25 per cent., use a 0·5000-g sample. For contents between 0·25 and 0·50 per cent., use a 0·2500-g sample, and add 0·2500 g of pure iron; alternatively, smaller cells can be used for optical-density measurements, but if this technique is adopted a calibration graph must be prepared with use of the smaller cells.
2. If insoluble matter, *e.g.*, silica or graphite, is present, filter the solution through a Whatman No. 40 filter-paper, wash the residue and filter-paper well with water, add the washings to the filtrate, and dilute to 100 ml in a calibrated flask.
3. It is always necessary to cool the solution, as heat is produced during the addition of the *NN*-dimethylformamide.
4. The presence of iron in the blank is essential, otherwise the optical density of the final solution will be variable. When measured against the compensating solution in 4-cm cells, an optical-density blank value of less than 0·025 should readily be achieved.

RESULTS

Table V shows the results obtained when the proposed procedure was applied to a series of plain-carbon and alloy steels and cast irons. Each replicate determination was carried out on a separate portion of sample and not on a different aliquot from a single solution; the results therefore indicate the over-all precision of the method.

CONCLUSIONS

It has been shown that 2,2′-biquinolyl can be used for determining copper in steels and cast irons without the necessity for a liquid - liquid extraction stage. Although, for a given copper content, the proposed method yields an optical density less than those obtained in certain procedures involving liquid - liquid extraction, this is due to the extent of final dilution and is of no consequence in ferrous analysis. The method is eminently suitable for routine determinations, and results are highly accurate.

TABLE V

COPPER CONTENTS FOUND IN CAST IRONS AND STEELS

Description of sample	Copper content (certificate value and range), %	Copper found by proposed method, %
B.C.S. No. 206; high silicon and phosphorus cast iron (grey)	0·02 (0·012 to 0·023)	0·023, 0·022, 0·026
B.C.S. No. 206/1; high silicon and phosphorus cast iron (grey)	0·05 (0·04 to 0·05)	0·046, 0·045, 0·049
B.C.S. No. 218/2; steel containing 0·15 per cent. of carbon	0·230 (not standardised)	0·240, 0·240, 0·230
B.C.S. No. 239/1; steel containing 0·3 per cent. of carbon	0·46 (0·45 to 0·47)	0·468, 0·470, 0·460
B.C.S. No. 274; mild steel	0·04$_0$ (0·037 to 0·043)	0·039, 0·038, 0·042
B.C.S. No. 221; steel containing 0·6 per cent. of carbon	0·09 (not standardised)	0·092, 0·093, 0·093
N.B.S. No. 16d; steel containing 1 per cent. of carbon	0·052 (0·046 to 0·056)	0·053, 0·053, 0·051
N.B.S. No. 101d; steel containing 18 per cent. of chromium and 9 per cent. of nickel	0·184 (0·18 to 0·191)	0·182, 0·185, 0·185
N.B.S. No. 121b; steel containing 18 per cent. of chromium and 11 per cent. of nickel	0·125 (0·12 to 0·131)	0·120, 0·120, 0·125
N.B.S. No. 160a; steel containing 19 per cent. of chromium, 14 per cent. of nickel and 3 per cent. of molybdenum	0·174 (0·169 to 0·19)	0·180, 0·177, 0·181
B.C.S. No. 272; mild steel	0·01$_5$ (0·014 to 0·016)	0·018, 0·017, 0·016
B.C.S. No. 230; chromium - molybdenum steel	0·085 (0·08 to 0·09)	0·084, 0·083
B.C.S. No. 273; mild steel	0·16$_0$ (0·150 to 0·170)	0·155, 0·158, 0·160
B.C.S. No. 235; stainless steel containing titanium and tungsten	0·98 (0·94 to 0·99)	0·988, 0·990, 0·992
B.C.S. No. 235/1; stainless steel containing titanium	0·13 (0·13)	0·129, 0·129, 0·131
B.C.S. No. 276; mild steel	0·12$_5$ (0·111 to 0·129)	0·125, 0·125, 0·125
B.C.S. No. 220; high-speed steel (tungsten - molybdenum)	0·14 (0·125 to 0·144)	0·142, 0·138, 0·142

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The Determination of Trace Impurities in High-purity Selenium

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Neutron-activation methods have been developed for the determination of trace amounts of cadmium, copper, nickel, tellurium and zinc in high-purity selenium. The methods have sensitivities much greater than those of other analytical procedures. The lower limits of determination are 0·005 p.p.m. of cadmium, 0·001 p.p.m. of copper, 0·05 p.p.m. of nickel and 0·01 p.p.m. of tellurium and zinc.

The current interest in the effects of impurities in semi-conductors has emphasised the need for determining trace impurities in these materials to well below the part per million level. Semi-conductors are of practical importance in a number of connections, such as transistors, crystal rectifiers, thermistors, phosphors and photoconductors, and the great sensitivity of their unique electrical properties to the presence of certain impurities has resulted in the development of new methods of purification. Consequent upon this there has been an ever-increasing use of neutron-activation analysis to determine trace amounts of impurities remaining in the purified products. This technique has been applied to the examination of high-purity products, and in this paper procedures for determining trace amounts of cadmium, copper, nickel tellurium and zinc in selenium are described.

The activation procedure consists in irradiating the sample in a flux of thermal neutrons and then dissolving the radioactive sample in the presence of inactive carriers of the trace elements sought. The carriers are separated from the matrix solution and radiochemically purified to separate the various activated impurities. The activity of each final precipitate is measured and compared with that of an appropriate standard.

Of the five trace impurities examined, nickel, when irradiated, gives only one convenient radioactive isotope, nickel-65, the half-life of which (2·56 hours) is sufficiently short to be a limiting factor. The procedure for radiochemical separation and purification must therefore be rapid. Irradiation of cadmium, copper, tellurium and zinc produces radioactive isotopes with longer half-lives, but, to attain maximum sensitivity, counting of the separated radioactive impurity has to begin on the day that the sample is removed from the atomic pile. As the amounts of impurities in the samples are near to the lower limits of determination, it is essential that the radiochemical purification should produce a product free from radioactive impurities in the shortest possible time. The radiochemical purity can be checked by examination of the decay curve and, when applicable, by gamma-ray scintillation spectrometry.

In order to relate the activity of the radio-isotope (or isotopes) contained in the separated carrier to the mass of its parent element in the sample, it must be compared with the activity of a standard (a known weight of the impurity element irradiated in the same container as the sample to ensure exposure to an identical flux of neutrons).

Experience has shown that it is preferable to determine only one element in one portion of sample. In this way, sources for radiochemical assay are separated with maximum yield in the shortest time. Composite schemes of separation are time-consuming and therefore often decrease the sensitivity.

Experimental

Samples of selenium in pellet form were sealed in polythene "lay-flat" tubing, packed in aluminium cans together with suitable standards (portions of the pure element to be determined) and irradiated in the BEPO pile at Harwell. The choice of irradiation conditions depends on the element being determined, but short periods of irradiation were favoured in order to keep the activity of the selenium matrix to a minimum. After irradiation, the sample was etched in dilute nitric acid to remove surface contamination and then dissolved in nitric acid, with the addition of an inactive carrier solution containing a known amount of the element being determined. The excess of nitric acid was removed by evaporation to dryness on a steam-bath, and the residue was dissolved in water.

To avoid escape of radioactive selenium into the atmosphere by volatilisation, hydrochloric acid was not used until the carrier had been separated from the matrix solution.[1] Removal of the matrix by reduction to elemental selenium with sulphur dioxide was unsatisfactory, since relatively large volumes of liquid were required for the reaction. As most of the chemical operations were carried out in 30-ml centrifuge tubes this greatly increased the time required to complete the separation and purification. In all determinations, therefore, the carrier containing the radioactive isotope or isotopes was precipitated, and the selenium was held in solution. When the carrier was free from selenium, a series of precipitation reactions was used to scavenge radioactive impurities.

Scavenged precipitates were separated by centrifugation, although it was occasionally necessary to filter the supernatant liquid to remove small particles before the subsequent purification stage. Finally, the carrier was precipitated for radioactive assay and collected on a 2-cm Whatman filter-paper of a suitable grade.

During the chemical operations the radioactive impurity accompanied the corresponding inactive carrier. Care was taken to ensure that both radioactive and inactive elements were in the same valency state during mixing. Loss of activity owing to incomplete separation was corrected for by determining the ratio of the initial and final amounts of carrier. The radio-isotopes used are listed below.

Element	Cadmium	Copper	Nickel	Tellurium	Zinc
Isotope used	Cadmium-115	Copper-64	Nickel-65	Tellurium-127	Zinc-69*m*
Half-life	2·3 days	12·8 hours	2·56 hours	9·3 hours	14 hours

RADIOACTIVE ASSAY—

Elemental standards in a coherent form were weighed on a Bunge microbalance before irradiation and irradiated together with the sample. After irradiation, a solution containing a known concentration of each standard was prepared. An aliquot of this solution (of size such as to give a count rate similar to that of the impurity isolated from the sample) was taken, inactive carrier was added, and the standard was subjected to the final precipitation stage of the chemical procedure as applied to the sample. The amount of carrier added was such that the precipitate from the standard was approximately equal in size to that previously obtained from the sample. Preparation of the standard was begun after the chemical separation of the impurity carrier had been completed, in order to avoid cross-contamination.

The final precipitates for radioactive assay were separated by filtration on 2-cm circles of filter-paper, which were subsequently placed in aluminium planchets, and the beta-particle activity was measured with a thin end-window Geiger tube. To eliminate, as far as possible, any errors arising from self-absorption in the precipitate or from variations in counting geometry, both sample and standard were mounted in the same chemical and physical form. It was arranged that their masses should be similar, and they were aligned with respect to the window of the Geiger tube by means of a Perspex former. Corrections were made to the observed count rate for the paralysis time of the counter and the background activity.

The possibility of serious errors in the count rates being caused by self-absorption of beta-particles in the final precipitates was examined. Five solutions, each containing radioactive isotopes of one of each of the elements cadmium, copper, nickel, tellurium and zinc were prepared. Aliquots (1 ml) of these solutions were added to carrier solutions containing increasing amounts of the corresponding inactive element. Precipitates of cadmium phosphate, cuprous thiocyanate, nickel dimethylglyoxime, tellurium and zinc mercuric thiocyanate were prepared, collected and counted as described under "Method." Decay curves were plotted, and the initial count rates were corrected for recovery of the carrier. The results are shown in Table I and indicate that the level of self-absorption of beta-particles by thick layers of precipitate was low in all the precipitates examined; self-absorption was therefore ignored.

METHOD

CARRIER SOLUTIONS—

Solutions of antimony, bismuth, cadmium, copper, iron, gold, manganese, mercury, nickel, selenium, tellurium and zinc each containing 10 mg of element per ml. Prepare the carrier solutions so that the elements they contain are in the same valency state as the corresponding elements in the sample solution.

PROCEDURE FOR CADMIUM—

Irradiate 1 g of sample at pile factor 10 (10^{12} neutrons per sq. cm per second) for 24 hours. Etch the sample in 4 N nitric acid, and dissolve in a mixture of 10 ml of concentrated nitric

acid and 1 ml of cadmium carrier solution. Evaporate to dryness on a steam-bath, and dissolve the residue in 15 ml of water. Add 2 drops of methyl red indicator solution, make just alkaline with 2 N sodium hydroxide, spin in a centrifuge, and discard the solution. Dissolve the precipitate in 1 ml of 5 N hydrochloric acid, dilute to 15 ml with water, make just alkaline with 2 N sodium hydroxide, spin in a centrifuge, and discard the solution.

TABLE I

RESULTS OF SELF-ABSORPTION STUDIES

Precipitate	Amount of carrier taken, mg	Activity of precipitate, counts per minute
Cadmium phosphate	5	1664
	10	1680
	15	1638
Cuprous thiocyanate	5	1115
	10	1150
	20	1066
Nickel dimethylglyoxime	3	2000
	6	2000
	9	1995
Tellurium	5	30,600
	10	30,500
	20	31,100
Zinc mercuric thiocyanate	5	5481
	7·5	5485
	10	5461

Dissolve the precipitate in 2 ml of concentrated hydrochloric acid, add 1 ml each of antimony and copper carrier solutions, dilute to 10 ml with water, and warm. Saturate with hydrogen sulphide, spin in a centrifuge, and discard the precipitate. To the solution add 1 ml of antimony carrier solution, saturate with hydrogen sulphide, spin in a centrifuge, and discard the precipitate. Filter the solution through a 9-cm Whatman No. 41 filter-paper, and evaporate the filtrate to expel hydrogen sulphide. Add 2 ml of iron carrier solution and 1 drop of 20-volume hydrogen peroxide, warm on a steam-bath, add 0·5 g of ammonium chloride, and make the solution ammoniacal. Warm until the precipitate coagulates, spin in a centrifuge, discard the precipitate, and filter the solution through a 9-cm Whatman No. 41 filter-paper. Make the filtrate just acid (approximately 0·1 N) with hydrochloric acid, warm, saturate with hydrogen sulphide, spin in a centrifuge, and discard the solution. To the precipitate add 10 ml of 1 N sodium hydroxide, warm, stir, spin in a centrifuge, and discard the solution. Add 10 ml of water, stir, spin in a centrifuge, and discard the solution.

Dissolve the precipitate in 2 ml of concentrated hydrochloric acid, and evaporate to dryness. Dissolve the residue in 10 ml of water, boil, filter if necessary, add 2 ml of a saturated solution of diammonium hydrogen orthophosphate, and allow to cool to room temperature, with occasional stirring. Separate the precipitate on a washed, dried and tared 2-cm Whatman No. 542 filter-paper, and wash it with water and then 95 per cent. ethanol. Dry to constant weight at 110° C, weigh, mount, and count at 24-hour intervals.

Convert the activity to weight of cadmium by comparison with a standard prepared as follows. Irradiate 0·1 mg of thin cadmium foil at the same time as the sample. Dissolve the foil in 2 ml of concentrated hydrochloric acid, and dilute to 100 ml with water. To a suitable aliquot add sufficient cadmium carrier solution to give a weight of final precipitate similar to that obtained from the sample. Dilute to 15 ml with water, warm, saturate with hydrogen sulphide, and spin in a centrifuge. Discard the solution, and continue as described for the sample in the preceding paragraph.

PROCEDURE FOR COPPER—

Irradiate 1 g of sample at pile factor 10 for 24 hours. Etch the sample in 4 N nitric acid, and dissolve in a mixture of 10 ml of concentrated nitric acid and 2 ml of copper carrier solution. Evaporate to dryness on a steam-bath, and dissolve the residue in 15 ml of water. Add 2 drops of methyl red indicator solution, make alkaline with 2 N sodium hydroxide, spin in a centrifuge, and discard the solution. Dissolve the precipitate in 4 ml of concentrated hydrochloric acid, add 1 ml each of antimony and mercury (mercuric) carrier solution, dilute to 15 ml with water, and warm. Saturate with hydrogen sulphide, spin in a centrifuge, and discard the solution. To the precipitate add 10 ml of 1 N sodium hydroxide, warm, with

stirring, spin in a centrifuge, and discard the solution. Add 5 ml of 8 N nitric acid, stir, warm on a steam-bath for 5 minutes, spin in a centrifuge, and discard the residue. Evaporate the solution to dryness, add 1 ml each of selenium and tellurium carrier solutions, and evaporate to dryness. Dissolve the residue in 3 ml of concentrated hydrochloric acid, dilute to 10 ml with water, saturate with sulphur dioxide, spin in a centrifuge, and discard the precipitate. Evaporate the solution to expel sulphur dioxide, and filter through a 7-cm Whatman No. 42 filter-paper.

To the filtrate add 1 ml each of antimony, cadmium and mercury (mercuric) carrier solutions, dilute to 15 ml with 3 N hydrochloric acid, and warm. Saturate with hydrogen sulphide, spin in a centrifuge, and discard the solution. To the precipitate add 10 ml of 1 N sodium hydroxide, warm, with stirring, spin in a centrifuge, and discard the solution. Add 5 ml of 8 N nitric acid, stir, warm on a steam-bath for 5 minutes, spin in a centrifuge, and discard the residue. To the solution add 1 ml of bismuth carrier solution, dilute to 10 ml with water, and make ammoniacal. Stir, spin in a centrifuge, and discard the precipitate. Acidify the solution with hydrochloric acid, warm, saturate with hydrogen sulphide, spin in a centrifuge, and discard the solution.

Dissolve the precipitate in 5 ml of 8 N nitric acid, and evaporate to dryness. Dissolve the residue in 2 ml of concentrated hydrochloric acid, evaporate nearly to dryness, dilute to 10 ml with water, and filter if necessary. Saturate the solution with sulphur dioxide, add 1 ml of a 5 per cent. solution of ammonium thiocyanate, and continue to pass sulphur dioxide until the precipitate is colourless. Separate the precipitate on a washed, dried and tared 2-cm Whatman No. 542 filter-paper, and wash it with water, ethanol and finally diethyl ether. Dry to constant weight at 110° C, weigh, mount, and count at 12-hour intervals.

Convert the activity to weight of copper by comparison with a standard prepared as follows. Irradiate 1 mg of copper at the same time as the sample. Dissolve this standard in 2 ml of concentrated nitric acid, evaporate to dryness, dissolve the residue in 5 ml of concentrated hydrochloric acid, and dilute to 100 ml with water. To a suitable aliquot add sufficient copper carrier solution to give a weight of final precipitate similar to that obtained from the sample. Dilute to 10 ml with water, warm, saturate with hydrogen sulphide, and spin in a centrifuge. Discard the solution, and continue as described for the sample in the preceding paragraph.

PROCEDURE FOR NICKEL—

Irradiate 1 g of sample at pile factor 10 for 6 hours. Etch the sample in 4 N nitric acid, and dissolve in a mixture of 10 ml of concentrated nitric acid and 1 ml of nickel carrier solution. Evaporate to dryness on a steam-bath, and dissolve the residue in 15 ml of water. Add 2 drops of methyl red indicator solution, make alkaline with 2 N sodium hydroxide, spin in a centrifuge, and discard the solution. Dissolve the precipitate in 1 ml of concentrated hydrochloric acid, dilute to 15 ml with water, make ammoniacal, and acidify to pH 4 with acetic acid. Add a slight excess of a 1 per cent. w/v solution of dimethylglyoxime in ethanol, stir, spin in a centrifuge, and discard the solution.

Dissolve the precipitate in 3 ml of concentrated nitric acid, evaporate to dryness, and bake the residue on a hot-plate.[2] Dissolve the residue in 5 ml of concentrated nitric acid, and dilute to 10 ml with water. Add 1 ml of manganese carrier solution, warm on a steam-bath, add 0·5 g of potassium bromate in small portions, and stir. Allow the precipitate to settle, spin in a centrifuge, and discard the precipitate. Repeat the scavenging step for manganese. Evaporate the solution to small volume, spin in a centrifuge, and discard any residue that separates. Dilute the solution to 15 ml with water, make alkaline with 2 N sodium hydroxide, spin in a centrifuge, and discard the solution. To the precipitate add 1 ml each of antimony, cadmium and copper carrier solutions, dissolve in the minimum volume of 3 N hydrochloric acid, and dilute to 15 ml with water. Warm, saturate with hydrogen sulphide, spin in a centrifuge, and discard the precipitate. Evaporate the solution to expel hydrogen sulphide, add 2 ml of iron carrier solution and 1 drop of 20-volume hydrogen peroxide, and warm on a steam-bath. Add 1 g of ammonium chloride, make ammoniacal, spin in a centrifuge, discard the precipitate, and filter the solution through a 9-cm Whatman No. 41 filter-paper.

Acidify the filtrate to pH 4 with acetic acid, add a slight excess of the dimethylglyoxime solution, and stir. Warm on a steam-bath for 5 minutes, spin in a centrifuge, and discard the solution. Add 10 ml of water to the precipitate, stir, and filter the mixture through a

washed, dried and tared 2-cm Whatman No. 541 filter-paper. Wash the precipitate with water, dry to constant weight at 110° C, weigh, mount, and count at 30-minute intervals.

Convert the activity to weight of nickel by comparison with a standard prepared as follows. Irradiate 1 mg of nickel at the same time as the sample. Dissolve it in 3 ml of 8 N nitric acid, evaporate to dryness, dissolve the residue in 5 ml of concentrated hydrochloric acid, and dilute to 100 ml with water. To a suitable aliquot add sufficient nickel carrier solution to give a final precipitate similar in weight to that obtained from the sample. Dilute to 15 ml with water, make ammoniacal, and continue as described for the sample in the preceding paragraph.

PROCEDURE FOR TELLURIUM—

Irradiate 1 g of sample at pile factor 10 for 24 hours. Etch the sample in 4 N nitric acid, and dissolve it in a mixture of 10 ml of concentrated nitric acid and 2 ml of tellurium carrier solution. Evaporate to dryness on a steam-bath, and dissolve the residue in 15 ml of water. Add 2 drops of methyl red indicator solution, make alkaline with 2 N sodium hydroxide, and acidify to pH 4 with acetic acid. Stir, spin in a centrifuge, and discard the solution. Dissolve the precipitate in 3 drops of concentrated hydrochloric acid, and repeat the precipitation of tellurium dioxide.

To the precipitate add a mixture of 10 ml of concentrated hydrochloric acid and 1 ml each of gold and selenium carrier solutions, warm, and allow to cool. Saturate with sulphur dioxide, spin in a centrifuge, and discard the precipitate. Dilute the solution to 25 ml with water, saturate with sulphur dioxide, spin in a centrifuge, and discard the solution. Wash the precipitate well with water, add 3 ml of concentrated nitric acid, warm on a steam-bath for 15 minutes, and cool. Dilute to 10 ml with water, filter through a 7-cm Whatman No. 44 filter-paper, and discard the residue. Make the filtrate alkaline with 5 N sodium hydroxide, acidify to pH 4 with acetic acid, stir, spin in a centrifuge, and discard the solution.

Dissolve the precipitate in 10 ml of concentrated hydrochloric acid, dilute to 25 ml with water, saturate with sulphur dioxide, and allow the precipitate to settle. Separate the precipitate on a washed, dried and tared 2-cm Whatman No. 542 filter-paper, and wash it with water, ethanol and finally diethyl ether. Dry to constant weight at 105° C, weigh, mount, and count at 9-hour intervals.

Convert the activity to weight of tellurium by comparison with a standard prepared as follows. Irradiate 1 mg of tellurium at the same time as the sample. Dissolve it in 3 ml of concentrated nitric acid, evaporate to dryness, cool, dissolve the residue in 2 ml of concentrated hydrochloric acid, and dilute to 100 ml with water. To a suitable aliquot add sufficient tellurium carrier solution to give a final precipitate similar in weight to that obtained from the sample. Dilute to 15 ml with water, make alkaline with 2 N sodium hydroxide, and acidify to pH 4 with acetic acid. Stir, spin in a centrifuge, discard the solution, and continue as described for the sample in the preceding paragraph.

PROCEDURE FOR ZINC—

Irradiate 1 g of sample at pile factor 10 for 24 hours. Etch the sample in 4 N nitric acid, and dissolve in a mixture of 10 ml of concentrated nitric acid and 1 ml of zinc carrier solution. Evaporate to dryness on a steam-bath, dissolve the residue in 5 ml of 4 N nitric acid, and dilute to 15 ml with water. Add 2 ml of a saturated solution of oxalic acid, stir, and then add 3 ml of potassium mercuric thiocyanate reagent solution (39 g of potassium thiocyanate and 27 g of mercuric chloride per litre). Stir until precipitation occurs, set aside for 15 minutes with occasional stirring, spin in a centrifuge, and discard the solution. Wash the precipitate with 10 ml of water, spin in a centrifuge, and discard the solution.

Dissolve the precipitate in 2 ml of concentrated nitric acid, and evaporate to dryness. Dissolve the residue in a mixture of 2 ml of concentrated hydrochloric acid and 1 ml each of cadmium and copper carrier solutions. Evaporate to dryness, dissolve the residue in 2 ml of concentrated hydrochloric acid, and evaporate nearly to dryness. Dilute to 15 ml with water, warm, saturate with hydrogen sulphide, spin in a centrifuge, and discard the precipitate. To the solution add 1 ml of antimony carrier solution, saturate with hydrogen sulphide, spin in a centrifuge, and discard the precipitate. Evaporate the solution to expel hydrogen sulphide, and add 1 ml of iron carrier solution, 1 drop of 20-volume hydrogen peroxide and 1 g of ammonium chloride. Warm, make ammoniacal, stir, spin in a centrifuge, and discard the precipitate. Filter the solution through a 9-cm Whatman No. 41 filter-paper, saturate the filtrate with hydrogen sulphide, spin in a centrifuge, and discard the solution. Dissolve

the precipitate in 2 ml of 8 N nitric acid, and evaporate to dryness. Dissolve the residue in 1 ml of concentrated nitric acid, dilute to 15 ml with water, and filter through a 9-cm Whatman No. 42 filter-paper.

To the filtrate add 2 ml of a saturated solution of oxalic acid and 3 ml of the potassium mercuric thiocyanate reagent solution. Stir until precipitation occurs, set aside for 15 minutes with occasional stirring, spin in a centrifuge, and discard the solution. Suspend the precipitate in 5 ml of water, and filter through a washed, dried and tared 2-cm Whatman No. 540 filter-paper. Wash the precipitate with water, ethanol and finally diethyl ether. Dry to constant weight at 110° C, weigh, mount, and count at 12-hour intervals.

Convert the activity to weight of zinc by comparison with a standard prepared as follows. Irradiate 1 mg of zinc at the same time as the sample. Dissolve it in 2 ml of 8 N nitric acid, and dilute to 100 ml with water. To a suitable aliquot add sufficient zinc carrier solution to give a final precipitate similar in weight to that obtained from the sample. Evaporate to dryness, dissolve the residue in 1 ml of concentrated nitric acid, dilute to 15 ml with water, and continue as described for the sample in the preceding paragraph.

Results

Two samples of high-purity selenium were examined by the proposed method. One was a commercial sample purchased from Mining and Chemical Products Ltd. and the other was a zone-refined specimen from the Royal Radar Establishment. The results are shown in Table II, together with the initial count rates of the separated impurities and the lower limits of determination.

Table II

Concentrations of impurities found in high-purity selenium

The background activity was approximately 7 counts per minute

Impurity determined	Sample No.*	Concentration of impurity found, p.p.m.	Initial count rate, counts per minute	Lower limit of determination, p.p.m.
Cadmium	1	0·052, 0·053, 0·048, 0·048, 0·048 (mean 0·050)	~90	0·005
	2	<0·005	< 7	
Copper	1	0·030, 0·034 (mean 0·032)	600	0·001
	2	0·190, 0·194 (mean 0·192)	3900	
Nickel	1	<0·05	< 7	0·05
	2	<0·05	< 7	
Tellurium	1	0·700, 0·740 (mean 0·720)	1400	0·01
	2	0·024, 0·028, 0·026 (mean 0·026)	~60	
Zinc	1	0·050, 0·043, 0·045 (mean 0·046)	~40	0·01
	2	0·017, 0·018 (mean 0·018)	~10	

* Sample No. 1 was obtained from Mining and Chemical Products Ltd. and sample No. 2 from the Royal Radar Establishment.

The results in Table II are the arithmetical means of replicate determinations on 1-g portions of sample. Each lower limit of determination was calculated from the initial count rate and the decay pattern of the isolated radioactive impurities and is taken as the concentration that will give an initial count rate approximately equal to the background count rate for a 1-g sample. The limit of determination for nickel was determined from the initial count rates of standards.

The work described was carried out as part of the general research programme of the National Physical Laboratory, and this paper is published by permission of the Director of the Laboratory.

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Colorimetric Determination of Uranium in Phosphate Rock after Extraction with Alkyl Acid Phosphates

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A method for determining uranium in phosphate rock and phosphoric acid has been developed. It is based on extraction of quadrivalent uranium by a solution of lauryl acid phosphates in light petroleum, this reagent being easily prepared from dodecanol and phosphorus pentoxide. The organic extract is evaporated to dryness, heated with nitric, sulphuric and perchloric acids and analysed colorimetrically with hydrogen peroxide.

Many phosphate rocks have uranium contents of the order of 0·01 per cent., and determinations are required to ascertain whether the rocks or the products formed in their chemical processing contain sufficient uranium to bring them within the scope of the Radioactive Substances Act, 1960. This act requires the registration of users of all substances containing more than 3×10^{-4} μc of uranium activity per g of a solid or 2×10^{-5} μc per g of a liquid. For uranium of natural isotopic composition, these figures correspond to approximately 0·05 per cent. of U_3O_8 in solids and 0·0033 per cent. in liquids. It must be borne in mind that phosphate rocks may contain radioactive daughters of uranium and also thorium and its radioactive daughters, but the implications of this cannot be discussed here. Determinations of uranium are also required in connection with processes for its removal from phosphates, this removal being effected either for the recovery of the valuable element or for the manufacture of pure phosphates.

Preliminary experiments

Solvent extraction—

The first step in the determination is to concentrate the uranium and separate it from most of the other constituents with which it is found. One of the best concentration techniques in trace-metal analysis is solvent extraction, and this was considered for our problem. Ethyl acetate and diethyl ether are widely used to extract uranium from aqueous solutions, as uranyl nitrate, but extraction is poor if anions other than nitrate are present, phosphate being particularly troublesome. Organic phosphates, such as tributyl phosphate,[1] are slightly better extractants than ethyl acetate or ether, but recovery of uranium is again poor in the presence of inorganic phosphate. The acid phosphates, such as dibutylphosphoric acid[2] and dilauryl dihydrogen pyrophosphate, have much higher distribution coefficients, and, as the extractants combine chemically with uranium,[3] the nature of the anion is comparatively unimportant. The phosphine oxides[4] would appear to be suitable extractants, but are not readily available, and we confined our studies to mixtures of acid phosphates, which can be easily prepared.

Dialkyl pyrophosphates have been recommended[5,6] for a commercial process for extracting uranium from phosphoric acid, but we have seen no reference to their use in analysis. For the commercial process, economic considerations led to the choice of compounds prepared from an octanol or from trimethylheptanol, but the cost of the alcohol used in analysis is insignificant, and we selected dodecanol, which was readily available in our laboratory. A few tests with 2-ethylhexanol yielded identical results, and it seems as though an acid phosphate mixture prepared from any fatty alcohol having 8 to 16 carbon atoms may be used in the analysis. We have made no attempt to determine the composition of the reagent and have accepted the description given in the papers referred to above (a dihydrogen pyrophosphate).

The reagent can be readily prepared from phosphorus pentoxide and dodecanol and is used as an approximately 0·01 M solution in light petroleum. We originally used ligroin, boiling range 100° to 120° C, as solvent, but considerable emulsification occurred, and light petroleum was finally selected after many alternative solvents had been investigated. Emulsification was further decreased by adding ethanol and sodium chloride to the aqueous solution. The reagent was first used at a concentration of 0·25 M, but a 0·01 M solution gave identical results and was easier to prepare and use.

Dilauryl dihydrogen pyrophosphate extracts uranium in the quadrivalent form, U^{4+}. Long, Ellis and Bailes[5] stated that sexavalent uranium, as UO_2^{2+}, was also extracted to a small extent, but we found that reduction to the quadrivalent state was essential for our purpose. Sodium dithionite was originally used as reducing agent, but hydroxylamine was later found to be effective and more convenient in use.

The reagent is not specific for uranium, and small amounts of iron, aluminium, cerium and calcium are present in the extracts from phosphate rocks. Iron was present only when the extraction with cupferron described below had been omitted. Aluminium passes with the uranium into the final solution, but it causes no error in the colorimetric peroxide method. Phosphate rocks rarely contain enough cerium to interfere with the determination of uranium, and the trace amounts found in Kola rocks are largely removed by a final filtration. Calcium, too, is subsequently removed by filtration, but precipitation of calcium sulphate may be a nuisance during the evaporation with nitric and sulphuric acids. The element can be largely removed by shaking the organic phosphate extracts with dilute sulphuric acid and then filtering the mixture, but we have found filtration to be more troublesome than the bumping caused by the presence of calcium sulphate. When the extracts were washed with hydrochloric acid, the calcium content was decreased to a certain extent, and this operation was included in the final method.

REGENERATION OF URANYL IONS—

In a proposed large-scale process,[5] uranium is recovered from the solvent by precipitation as tetrafluoride. In trace analysis such a precipitation step may cause errors, and recovery by extraction with aqueous ammoniacal hydrogen peroxide was investigated; however, no immediate success was achieved. The alternative of evaporating the solvent and then destroying organic matter by wet oxidation proved to be convenient and was therefore adopted.

COLORIMETRIC DETERMINATION—

The solvent-extraction step readily allows preparation of a solution containing up to several milligrams of uranium in a few millilitres, and we were therefore not restricted to the use of sensitive methods for determining the uranium. The comparatively insensitive peroxide method[7] was selected because it is subject to few interferences. Tests showed that the presence of reasonable amounts of phosphate, sulphate and perchlorate could be tolerated, and filtration of the carbonate solution removed iron and several other interfering cations. The only elements thought likely to interfere are chromium, molybdenum and vanadium, but in phosphate rock these occur in such small amounts that serious interference is improbable.

In some instances, the solution was pale yellow before the hydrogen peroxide was added, and this was liable to cause erroneously high results. Error from this source was overcome by measuring the optical density of the solution before and after hydrogen peroxide was added and then using the difference as a measure of the uranium content.

A mercury-vapour lamp with a filter combination selecting radiation at 4047 A[8] is not an intense source of light, and better precision was achieved by balancing the instrument at a drum reading of 0·5 instead of the more usual 1·0. This decreased the range of the method, but, for high contents of uranium, the setting could easily be re-adjusted to 1·0; the lower precision was tolerable in such determinations, as the error is small in proportion to the results.

Although our work was done with a Spekker absorptiometer, there is no reason why a spectrophotometer should not be used, a wavelength in the range 3750 to 4100 A being suitable. If the cell has a capacity of less than 25 ml it may be more convenient to add the hydrogen peroxide to a separate portion of the prepared solution instead of to the contents of the cell used for setting the instrument as described under "Procedure for Phosphate Rock."

EXTRACTION WITH CUPFERRON—

A preliminary method for determining uranium was devised in accordance with the considerations described above and was used satisfactorily for many analyses. However, with certain samples having high contents of iron, the final filtration was somewhat troublesome, and prior removal of iron by extraction with cupferron and chloroform was investigated. This was found to give more precise results, even with samples low in iron. Cupferron extracts iron, titanium and vanadium from oxidised acid solutions,[7] and removal of these or other rare metals presumably effected the improvement. We therefore introduced

an extraction with cupferron into all determinations. The sample must be oxidised before extraction, otherwise some quadrivalent uranium may be lost as its cupferron complex; potassium chlorate was selected as oxidising agent.

Method

Reagents—

Hydrochloric acid, sp.gr. 1·18.

Potassium chlorate.

Cupferron solution, 1 per cent., aqueous—This solution should be freshly prepared and filtered before use.

Chloroform.

Hydroxylamine hydrochloride solution, 10 per cent. w/v, aqueous.

Sodium chloride.

Ethanol or industrial methylated spirit.

Sulphuric acid, 18 N.

Perchloric acid, 60 per cent. w/v.

Nitric acid, sp.gr. 1·42.

Sodium hydroxide, 5 N.

Hydrogen peroxide solution, 6 per cent. w/v.

Extraction solution—Weigh 1·00 g of lauryl alcohol (n-dodecanol) into a dry 150-ml beaker, and dissolve it in 100 ml of light petroleum, boiling range 40° to 60° C. Add an estimated 1 g of phosphorus pentoxide, exposing it to the air for the least possible time to minimise absorption of moisture. Set aside for 10 minutes, with occasional stirring, decant the solution from the excess of phosphorus pentoxide, and dilute to 250 ml with light petroleum. The solution is almost colourless at first, but darkens through yellow to brown on standing for 2 to 3 hours; it should be freshly prepared each day.

Standard uranium solution—Accurately weigh about 0·1 g of uranyl acetate dihydrate, dissolve in water, and dilute to 250 ml in a calibrated flask. This solution contains 2·65 w mg of U_3O_8 per ml, where w is the weight of uranyl acetate taken, in grams.

Procedure for phosphate rock—

Solution of sample—Accurately weigh about 5 g of sample into a 150-ml beaker, and add 15 ml each of water and hydrochloric acid, sp.gr. 1·18. Boil gently until the sample has dissolved, apart from small amounts of organic and siliceous matter. Add about 1 g of potassium chlorate in approximately 0·2-g portions, and boil after each addition. Add 20 ml of water, and filter the mixture through an 11-cm Whatman No. 40 filter-paper into a 250-ml separating funnel. Transfer the residue to the filter-paper with a jet of warm water, and wash it twice with warm approximately 0·5 N hydrochloric acid (2 ml of concentrated acid *plus* 40 ml of warm water). When analysing a new type of rock, it is necessary to ensure that no uranium remains in the residue (see p. 183).

Extraction of iron, etc.—Cool the solution in the separating funnel, dilute to about 100 ml, add 20 ml of 1 per cent. cupferron solution, and swirl. Add 25 ml of chloroform, and shake for 30 seconds; the brown precipitate of iron dissolves in the chloroform to give a deep-red solution. Allow the layers to separate, and run off the organic layer. Add 1 ml of cupferron solution; if a brown precipitate forms, add a further 4 ml of cupferron solution, and extract with 10 ml of chloroform. Discard the chloroform layer, and repeat the treatment with cupferron solution and chloroform until no brown precipitate forms when 1 ml of cupferron solution is added; this indicates that all the iron has been removed. Add 10 ml of chloroform, shake, and allow to separate (both layers should be almost colourless). Discard the chloroform layer.

Extraction of uranium—To the aqueous solution in the separating funnel add 5 ml of 10 per cent. hydroxylamine hydrochloride solution, 10 g of sodium chloride and 50 ml of ethanol or industrial methylated spirit, and swirl to dissolve. Add 25 ml of extraction solution, shake vigorously for 1 minute, and allow the layers to separate. Run the aqueous layer into a second separating funnel, and extract again with 15 ml of extraction solution. Combine the light petroleum layers, and wash with a mixture of 2·5 ml of hydrochloric acid, 15 ml of water, 10 ml of ethanol or industrial methylated spirit and 0·5 ml of hydroxylamine

hydrochloride solution. Discard the wash solution, run the light petroleum solution into a 100-ml beaker, introduce a boiling rod, cover with a watch-glass, and evaporate to dryness; alternatively, a small Kjeldahl flask can be used. Add 3 ml of 18 N sulphuric acid and 2 ml of nitric acid, sp.gr. 1·42, and evaporate until charring begins. Add nitric acid dropwise, with continued heating, until the organic matter is destroyed (usually about 5 ml of nitric acid are needed). To the residue add 2 ml each of nitric acid and 60 per cent. perchloric acid; use these acids to rinse the walls of the beaker or flask as thoroughly as possible. Evaporate until dense fumes are evolved, heat for a further 5 minutes, and allow to cool. Rinse the walls of the beaker or flask with about 10 ml of water, boil for a few minutes to expel chlorine, and cool.

Development of colour—Slowly add 5 N sodium hydroxide until the mixture is just alkaline to litmus paper. (Do not add the litmus paper until the solution has been partly neutralised or the paper will be disintegrated by the acid.) Add N sulphuric acid dropwise until the solution is just acid to litmus paper. Add 10 ml of M sodium carbonate, heat to boiling, digest at just below the boiling-point for 5 minutes, and filter the hot solution through a 12·5-cm Whatman No. 42 filter-paper into a 50-ml calibrated flask. Wash the beaker and filter-paper twice with a little warm water, and add the washings to the contents of the calibrated flask. Add 1 ml of 5 N sodium hydroxide, cool, dilute to the mark, and mix (the solution should be almost colourless).

By pipette, place 25 ml of the solution in a 4-cm absorptiometer cell, after first rinsing the cell and the pipette with a little of the solution. Set the Spekker absorptiometer to read 0·500 with water; use the mercury-vapour lamp and No. 1 and OV1 filters. Measure the optical density of the sample solution, place 1 ml of hydrogen peroxide solution in the cell by pipette, and stir to mix (a yellow colour is formed). Again measure the optical density, ensuring that no bubbles of oxygen are adhering to the walls of the cell. Finally, thoroughly rinse the cell with water to remove all traces of peroxide before the next determination is made.

Calculation—Convert the difference, D, between the optical-density readings before and after the addition of peroxide to milligrams of U_3O_8 by reference to a previously prepared calibration graph. Calculate the uranium content of the sample from the expression—

$$\text{Uranium content, as } U_3O_8, \% = \frac{\text{Weight of } U_3O_8, \text{ mg} \times 100}{\text{Weight of sample, g} \times 1000}$$

Preparation of calibration graph—By pipette, place a series of volumes, *e.g.*, 1, 3, 5 and 7 ml, of standard uranium solution in separate 50-ml calibrated flasks. To the contents of each flask add about 25 ml of water, 10 ml of M sodium carbonate and 1 ml of 5 N sodium hydroxide, swirl to mix, dilute to the mark, and mix again. Measure the optical density of each solution as described above, and plot a graph of values of D against the number of milligrams of U_3O_8 present. This should be a straight line, the weight of U_3O_8 being approximately equal to $D \times 4$.

PROCEDURE FOR CRUDE PHOSPHORIC ACID—

Accurately weigh about 5 g of sample into a small beaker, add 15 ml of hydrochloric acid, sp.gr. 1·18, and 0·5 g of potassium chlorate, heat to boiling, and boil gently for 5 minutes. Transfer to a 250-ml separating funnel, rinse the beaker with about 80 ml of water, and add the rinsings to the contents of the separating funnel. Continue as described above for phosphate rock, beginning at "Extraction of iron, etc."

INVESTIGATION OF THE METHOD

TESTS OF COLORIMETRIC FINISH—

Solutions containing 1·78 mg of U_3O_8, added as uranyl acetate, and various amounts of mineral acids and sodium salts were prepared. Colours were developed and measured as described under "Development of colour," but the adjustment of acidity with N sulphuric acid and the subsequent filtration were omitted.

The results are shown in Table I and indicated that sulphate and perchlorate decreased the differences, whereas phosphate increased them; when the anions were mixed the effects tended to cancel one another. This was further shown by the calibration results in Table II, where the optical-density differences found in the last series of experiments (in which the

anion contents resemble those obtained in practice) agree well with those found when no extra anions were present.

TABLE I

EFFECTS OF ANIONS

Each solution contained 1·78 mg of U_3O_8

Substances added	Optical-density difference
None	0·423
Na_2SO_4 (5 g)	0·393
Sulphuric acid, 18 N (3 ml)	0·390
Perchloric acid (3 ml)	0·378
$NaH_2PO_4.2H_2O$ (0·15 g)	0·440
$NaH_2PO_4.2H_2O$ (0·30 g)	0·450
Sulphuric acid, 18 N (3 ml) *plus* $NaH_2PO_4.2H_2O$ (0·15 g)	0·421

TABLE II

EFFECTS OF ANIONS ON CALIBRATION GRAPHS

Substances added	Optical-density difference in presence of— 0·27 mg of U_3O_8	0·54 mg of U_3O_8	0·81 mg of U_3O_8	1·08 mg of U_3O_8	1·35 mg of U_3O_8	1·89 mg of U_3O_8
None	0·070	—	0·196	—	0·328	0·463
Na_2SO_4 (5 g) *plus* $NaH_2PO_4.2H_2O$ (0·10 g)	0·068	—	0·196	—	0·332	0·471
Na_2SO_4 (5 g) *plus* $NaH_2PO_4.2H_2O$ (0·15 g)	0·068	0·136	0·203	0·267	0·338	0·471
Na_2SO_4 (5 g) *plus* $NaH_2PO_4.2H_2O$ (0·15 g) *plus* $HClO_4$ (2 ml)	0·067	0·130	0·195	0·263	0·331	0·467

The results of further studies (see Table III) showed that uranium was lost during filtration if free sodium hydroxide was present. The experiments were carried out in the same way as those of Table I, except that all solutions were filtered and the acidity was adjusted with N sulphuric acid in experiments Nos. 3 and 4.

In experiments Nos. 1 and 2 the excess of 5 N sodium hydroxide amounted to only 1 or 2 drops, but even this small amount caused appreciable loss of uranium. The optical-density differences in experiments Nos. 3 and 4 agree well with the corresponding values in Table I, indicating that no uranium was lost during filtration when an excess of sodium hydroxide was avoided. The filtered solution contains a trace of bicarbonate, which is believed to cause slight variations in the intensity of the final yellow colour; a 1-ml excess of sodium hydroxide is therefore added after filtration.

TABLE III

EFFECT OF ALKALINITY AT FINAL FILTRATION

Each solution contained 1·78 mg of U_3O_8

Experiment No.	Substances added	Optical-density difference
Excess of sodium hydroxide present—		
1	Sulphuric acid, 18 N (3 ml)	0·139
2	Perchloric acid (3 ml)	0·176
Excess of sodium hydroxide absent—		
3	Sulphuric acid, 18 N (3 ml)	0·396
4	Sulphuric acid, 18 N (3 ml) *plus* $NaH_2PO_4.2H_2O$ (0·15 g)	0·423

TESTS FOR COMPLETENESS OF EXTRACTION—

A known amount of uranyl acetate equivalent to about 1·3 mg of U_3O_8 was added to each of several 3-ml portions of analytical-reagent grade orthophosphoric acid, and each mixture was diluted to 100 ml in a separating funnel. Certain additions were made to some of the mixtures, and the uranium contents were determined as described for phosphate rocks, beginning at "Extraction of uranium." The recoveries are shown in Table IV and averaged about 99 per cent., indicating that two extractions with the alkyl acid phosphates were sufficient; the presence of fluoride appeared to cause no difficulty.

TABLE IV

COMPLETENESS OF EXTRACTION

Experiment No.	Substances added	U_3O_8 present, mg	U_3O_8 found, mg	Recovery, %
1	None	1·335	1·32	99
2	None	1·325	1·32	100
3*	None	1·355	1·39	103
4	NaF (0·25 g)	1·325	1·34	101
5	$FeSO_4.7H_2O$ (0·01 g)	1·335	1·30	97
6	$FeSO_4.7H_2O$ (0·01 g)	1·350	1·35	98
7*	$FeSO_4.7H_2O$ (0·03 g)	1·355	1·28	94
8	$FeSO_4.7H_2O$ (0·03 g)	1·325	1·28†	96
9*	$FeSO_4.7H_2O$ (0·03 g)	1·355	1·35†	100

* Extraction solution used was prepared from 2-ethylhexanol instead of n-dodecanol.
† Determination begun at "Extraction of iron, etc.," *i.e.*, extraction with cupferron was included.

TESTS FOR COMPLETENESS OF SOLUTION OF SAMPLE—

In the procedure for phosphate rocks, the sample solution is filtered into a separating funnel and the residue of organic and siliceous matter is discarded. In some analyses, the filter-paper containing the insoluble matter was ignited (often two or three residues were ignited together) and the ash was evaporated with hydrofluoric and sulphuric acids. The residue was heated with 1 or 2 ml of sulphuric acid and then analysed for uranium as described, beginning at "Development of colour."

Tests on insoluble matter *plus* added uranyl salt showed that no uranium was lost during evaporation with hydrofluoric and sulphuric acids, but that, for precise results, the concentrations of anions in the final solution must be similar to those present in the solutions used for preparing the calibration graph.

No uranium was found in the insoluble residues from Moroccan rock, except in some early determinations when potassium chlorate was omitted, up to 0·002 per cent. of U_3O_8 then being found; the insoluble residue in the final method can therefore be discarded without loss of uranium. No uranium was found in the residue from Nauru rock, but with Florida rock the insoluble fraction contained U_3O_8 to the average extent of 0·0010 per cent. of the rock, and this is too much to be neglected. For Florida rocks, therefore, and all others for which complete solution of uranium has not been established, the recommended method of analysis is to treat the insoluble residue as described above, dissolve it in hydrochloric acid instead of sulphuric acid and then add the solution to the main filtrate in the separating funnel.

RESULTS

Samples of crude phosphoric acid (prepared by decomposing Moroccan phosphate rock with sulphuric acid and removing calcium sulphate by filtration) and various phosphate rocks were analysed by the proposed method; the results are shown in Table V. Most of the

TABLE V

TYPICAL RESULTS FOR CRUDE PHOSPHORIC ACID AND PHOSPHATE ROCKS

Sample	Approximate phosphate content, as P_2O_5, %	Uranium found, as U_3O_8, %
Phosphoric acid (May 1st, 1960)	—	0·0119, 0·0116
Phosphoric acid (September 25th, 1960) ..	26	0·0101, 0·0111, 0·0104, 0·0112
Moroccan phosphate (April 8th, 1960)	33	0·0134
Moroccan phosphate (April 12th, 1960) ..	33	0·0136, 0·0130, 0·0131, 0·0128
Moroccan phosphate (July 6th, 1960)	33	0·0131, 0·0133
Kola phosphate	39	0·0003
Negev phosphate	38	0·0054
Nauru phosphate	39	0·0071
Taiba phosphate	38	0·0103 *plus* 0·0003*

* The second figure is the amount of uranium found in the insoluble residue.

rocks other than Moroccan were produced by concentration of run-of-mine materials, and the uranium contents must not be regarded as typical of the deposits.

Kola phosphate is an igneous rock, whereas the others are sedimentary, and it is of interest to note that this contains little, if any, uranium. The result of 0·0003 per cent. is not significantly different from zero, and it would normally be checked by using a larger weight of sample or a more refined procedure.

A final check on the method was made by analysing certain samples to which known amounts of uranium had been added. Each addition consisted of uranyl acetate solution equivalent to 0·553 mg of U_3O_8 and was made to the filtered solution before extraction of iron. This corresponds to 0·0111 per cent. of U_3O_8 in a 5-g sample, and the results for "U_3O_8 expected" in Table VI are derived by adding this figure to the results shown for the rock in Table V.

Table VI

Results for phosphate rocks with added uranium

Each sample (5 g) contained 0·0111 per cent. of added U_3O_8

Sample	U_3O_8 expected, %	U_3O_8 found, %
Florida phosphate	0·0203	0·0202
Moroccan phosphate	0·0242	0·0234, 0·0245
Nauru phosphate	0·0182	0·0185
Taiba phosphate	0·0214	0·0214

From the results in Tables V and VI, the over-all standard error was calculated to be $\pm$0·00038 per cent. of U_3O_8, based on 13 degrees of freedom. The 95 per cent. confidence limits for a single determination are therefore estimated as approximately $\pm$0·0008 per cent. of U_3O_8.

Conclusions

A reagent prepared from dodecanol and phosphorus pentoxide is excellent for extracting uranium before its colorimetric determination. The proposed method is for phosphate rock and phosphoric acid, but there is no reason why it should not be applied to any other material; by taking larger weights of sample, uranium contents down to 10 p.p.m. could easily be determined. Alternatives to the colorimetric peroxide finish can be used, provided that the small amount of phosphate can be tolerated, and these should extend the range of applications of the procedure.

Another possibility would be to take the solution after destruction of organic matter, which contains only 0·03 g of phosphoric acid, and then carry out further separation by a more specific organic extractant; with such a procedure, the preliminary cupferron extraction would probably be unnecessary.

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A Colorimetric Procedure for Checking the Palladium Content of Jewellery Alloys

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In Atkinson's method for determining palladium by titration with potassium iodide, the end-point is detected by spinning in a centrifuge to settle the precipitated palladous iodide and then testing for excess of palladium in the supernatant solution by adding more potassium iodide and observing any "cloud" of palladous iodide formed.

In the proposed method, after most of the palladium has been precipitated with iodide and the solution spun in a centrifuge, the residual palladium is detected colorimetrically with *p*-nitrosodimethylaniline. The method would be suitable for adoption as a "pass or fail" test in a hall-marking assay context.

In recent years the possibility of hall-marking palladium alloys has been discussed, and in this connection the availability of a rapid procedure for confirming the nominal palladium contents of various jewellery alloys would be of importance. Atkinson[1,2] has described a volumetric method for determining palladium based on precipitation of palladous iodide from a palladium solution by a standard solution of potassium iodide. The solution is spun in a centrifuge, and the presence of residual palladium is detected by the formation of a "cloud" of palladous iodide after addition of further potassium iodide solution to the supernatant liquid. The point at which no cloud can be detected is taken as the end-point. The method can be adopted for use as a "pass test" for alloys of a given nominal composition, and in this form would be convenient for hall-marking assay purposes. For this purpose a calculated volume of potassium iodide solution is added to the solution of the alloy, so that all but a small proportion of the total palladium nominally present is precipitated. After centrifugation, the presence of residual palladium is detected by the formation of a cloud when a further drop of potassium iodide solution is added, thus confirming that the palladium content of the alloy is not less than the nominal.

In tests of Atkinson's procedure, observation of the faint cloud was sometimes difficult, and interpretation varied with different observers. If the residual palladium is determined colorimetrically with *p*-nitrosodimethylaniline,[3] the results obtained are considerably more reliable and reproducible. This paper describes the adaptation of the procedure to a "pass - fail" application.

Method

Reagents—

p-*Nitrosodimethylaniline solution*—A solution of 0·25 g of *p*-nitrosodimethylaniline in 100 ml of a mixture of equal volumes of water and ethanol (industrial methylated spirit).

Buffer solution—A mixture of 48 ml of N hydrochloric acid and 132 ml of N sodium acetate is diluted to 200 ml with water.

Standard palladium solution, 5 p.p.m.—Exactly 10 mg of pure palladium foil or sponge are dissolved in aqua regia, and the solution is evaporated twice to dryness with a few drops of hydrochloric acid. The residue is dissolved in a few drops of hydrochloric acid and diluted to 2 litres with water in a calibrated flask.

Ferrous sulphate solution, 20 per cent. w/v—Ferrous sulphate (200 g) is dissolved in a mixture of water and 50 ml of concentrated sulphuric acid, and the solution is diluted to 1 litre with water. The water used should be de-aerated by boiling or by passing a current of nitrogen through it. The solution is kept in a well-stoppered bottle.

Potassium iodide, 0·01 N—Potassium iodide (3·124 g) and 0·2 g of sodium carbonate are dissolved in water, and the solution is diluted to 2 litres. This solution, which should be stored in an amber-glass bottle, is standardised as described below.

A solution containing exactly 750 mg of palladium per litre is prepared by dissolving the requisite amount of pure palladium in aqua regia, evaporating to dryness with hydrochloric acid, dissolving the residue in water and diluting to standard volume.

Twenty-millilitre aliquots (≡ 15 mg of palladium) are treated with 15 ml of 20 per cent. w/v ferrous sulphate solution and then shaken with different carefully measured volumes of potassium iodide solution. The volumes of potassium iodide solution added to each aliquot are calculated from the approximate palladium equivalent, so that after centrifugation they yield a series of clear solutions with residual palladium contents in steps of 0·5 per cent. of the original palladium content, covering a short range on either side of the selected concentration of the comparison standard. By pipette, 1-ml portions of these solutions are placed in test-tubes and treated with 5 ml of buffer solution and then 0·25 ml of *p*-nitrosodimethylaniline solution. The colours developed are compared with the standard colour produced from 0·4 ml of standard palladium solution treated exactly as described under "Procedure."

The volume of potassium iodide solution to be taken as equivalent to 15 mg of palladium is that associated with the test sample that lies just above the standard in order of colour intensity. This method of deriving a factor for the potassium iodide automatically provides for the required slight excess of palladium in test solutions from alloys whose compositions correspond to the nominal.

PROCEDURE—

An accurately weighed sample of alloy containing approximately 15 mg of palladium is dissolved in aqua regia (9 drops of hydrochloric acid and 3 drops of nitric acid), and the solution is evaporated to dryness. The residue is dissolved in 15 ml of N hydrochloric acid. If necessary, the solution is filtered to remove any silver chloride, molybdic acid or tungstic acid and is then transferred to a glass-stoppered bottle of capacity about 150 ml. The solution is treated with 15 ml of 20 per cent. w/v ferrous sulphate solution, and a calculated volume of 0·01 N potassium iodide is added from a burette to give a small residual trace of unprecipitated palladium. The bottle is shaken thoroughly and then set aside for 10 to 15 minutes.

A portion of the contents is then spun in a centrifuge. By pipette, 1 ml of the clear supernatant liquid is placed in a 6-inch × ½-inch test-tube and treated with 5 ml of buffer solution and 0·25 ml of *p*-nitrosodimethylaniline solution.

In the final colorimetric comparison between test solution and the standard solution it was found that small differences in colour are most readily observed when the final solutions contain 0·1 to 0·2 p.p.m. of palladium.

Consequently, the colour developed is compared with a standard colour prepared concurrently by measuring with a pipette 0·4 ml of the standard palladium solution, adding 0·15 ml of 20 per cent. w/v ferrous sulphate solution, diluting to 1 ml with water and then treating with buffer solution and the colorimetric reagent solution. If the colour developed by the test solution is more intense than that of the standard the sample can be considered to have a palladium content not less than the nominal.

By comparison with suitable standard colours an estimate can be made of the difference, if any, between the palladium content and the nominal content.

The test may also be carried out by comparison of the colour developed in the test solution with that produced by carrying out the full procedure on an alloy of known palladium content, preferably an alloy of the nominal composition of the test sample. This method would be particularly suited to the hall-marking of a relatively large number of alloy samples of the same nominal palladium content.

INTERFERENCES

Under the conditions of the procedure, the method was accurate to 0·5 per cent. of palladium content in the presence of ruthenium, copper, tin, silver, aluminium, iron, magnesium, tungsten, platinum, gold and rhodium. As noted by Atkinson, the potential interference from platinum and gold was obviated by the presence of ferrous sulphate.

The method was assessed as a "pass" test as described below.

Measured volumes of a standard palladium solution, each containing exactly 15 mg of palladium, were treated with volumes of standard potassium iodide solution equivalent to the palladium present. Further volumes of palladium solution, also containing 15 mg of palladium each, were treated with volumes of potassium iodide solution equivalent to 0·5 per cent. more palladium than was present. The colours developed by the test procedure were compared with the standard prepared as described, and the samples were designated as

TABLE I

ASSESSMENT OF THE METHOD AS A "PASS" OR "FAIL" TEST

Reference code	Sample	Nominal palladium content,* %	Observed order of colour intensity†	Result indicated
A_1	Pure palladium	100·0	A_1; S; A_2	Pass
A_2		100·5		Fail
B_1	Pure palladium	100·5	B_2; S; B_1	Fail
B_2		100·0		Pass
C_1	Pure palladium	100·0	C_1; S; C_2	Pass
C_2		100·5		Fail
D_1	Pure palladium	100·5	D_2; S; D_1	Fail
D_2		100·0		Pass
E_1	95·5 palladium - 4·5 nickel	95·5	$E_1 = E_2$; S; $E_3 = E_4$	Pass
E_2		95·5		Pass
E_3	95 palladium - 5 nickel	95·5		Fail
E_4		95·5		Fail
F_1	95·5 palladium - 4·5 nickel	95·5	F_2; F_1; S; F_4; F_3	Pass
F_2		95·5		Pass
F_3	95 palladium - 5 nickel	95·5		Fail
F_4		95·5		Fail
G_1	95·5 palladium - 4·5 nickel	95·5	$G_1 = G_2$; S; $G_3 = G_4$	Pass
G_2		95·5		Pass
G_3	95 palladium - 5 nickel	95·5		Fail
G_4		95·5		Fail

* Refers to the palladium content on which the volume of potassium iodide solution was calculated, *e.g.*, D_1 will be as if it were 0·5 per cent. deficient in palladium.
† The deepest colour is given first; S represents the standard colour.

TABLE II

ASSESSMENT OF THE METHOD AS APPLIED TO A 95·5 per cent. PALLADIUM - 4·5 per cent. RUTHENIUM JEWELLERY ALLOY

Reference code	True sample weight, g	Nominal sample weight, g	Observed order of colour intensity*	Result indicated
J_1	0·01479	0·01487	J_3; J_4; S = J_1; J_2	Fail
J_2	0·01514	0·01522		Fail
J_3	0·01492	0·01492		Pass
J_4	0·01448	0·01448		Pass
K_1	0·01596	0·01596	K_1; K_2; K_4; S = K_3; K_5	Pass
K_2	0·01578	0·01578		Pass
K_3	0·01459	0·01467		Fail
K_4	0·01416	0·01416		Pass
K_5	0·01485	0·01493		Fail
L_1	0·01416	0·01416	L_1; L_2; L_3; S; L_4; L_5	Pass
L_2	0·01494	0·01494		Pass
L_3	0·01446	0·01446		Pass
L_4	0·01450	0·01458		Fail
L_5	0·01423	0·01431		Fail
M_1	0·01491	0·01499	M_3; M_4; S = M_1 = M_2	Fail
M_2	0·01470	0·01478		Fail
M_3	0·01468	0·01468		Pass
M_4	0·01382	0·01382		Pass
N_1	0·01610	0·01610	N_1; S = N_2; N_3; N_4	Pass
N_2	0·01436	0·01444		Fail
N_3	0·01436	0·01444		Fail
N_4	0·01602	0·01610		Fail
O_1	0·01731	0·01739	O_3; O_4; S; O_1; O_2; O_5	Fail
O_2	0·01707	0·01715		Fail
O_3	0·01632	0·01632		Pass
O_4	0·01438	0·01438		Pass
O_5	0·01650	0·01658		Fail

* The deepest colour is given first; S represents the standard colour.

"pass" or "fail" according to whether the colours were, respectively, more or less intense than the standard colour. The results of these experiments are shown in Table I. A similar procedure was used with palladium - nickel alloys differing in palladium content by 0·5 per cent., the amounts of potassium iodide solution to be added being calculated on a basis of the content of the richer alloy. The results of these experiments are also shown in Table I. All the results recorded in Table I gave a correct result as a "pass" or "fail" test.

Similarly, as a further test of the method, a first observer weighed out a series of samples of 95·5 per cent. palladium - 4·5 per cent. ruthenium jewellery alloy, each of about 15 mg. In some tests the recorded weight was made 0·08 mg greater than the true weight, thus making such samples 0·5 per cent. deficient in palladium. A second observer added the calculated volumes of potassium iodide solution based on the recorded weights and carried out the testing. The results are shown in Table II. With the criterion that for a "pass" the colour of the test samples must be perceptibly more intense than that of the standard, these results are also correct.

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The Determination of Cobalt in Heat-resistant Alloys*

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A method for determining cobalt in a wide variety of refractory alloys is discussed. The difficulties usually encountered in most methods (caused by interference from many elements) have been overcome by the specific precipitation of cobalt by a combination of phenylthiohydantoic and thioglycollic acids. The precipitated cobalt is accompanied only by trace amounts of iron and nickel, which offer no hindrance to the subsequent direct potentiometric titration with potassium ferricyanide solution in the presence of ethylenediamine. Cobalt has been successfully determined in iron-, cobalt- and nickel-based alloys containing various combinations of aluminium, chromium, copper, iron, manganese, molybdenum, nickel, niobium, silicon, tantalum, titanium, tungsten, vanadium and zirconium. For alloys containing more than 3 per cent. of cobalt, consistent results with errors of less than 2 to 4 per cent. can be expected. The method is considered suitable for umpire purposes. A standard potentiometric-titration apparatus or a pH meter with platinum and calomel electrodes may be used.

The choice of a method for determining higher amounts of cobalt in heat-resistant alloys is not always easy. A search of the chemical literature will reveal several methods and combinations of methods, but alloys are frequently encountered that do not respond well to the usual procedures unless extensive preliminary separations are devised. This makes the determination a relatively long and expensive operation; further, there is considerable room for improvement in the accuracy of most methods for cobalt. With this in mind, many existing procedures for determining cobalt were studied with the intention of combining some of them, if possible, into an improved procedure that could be used for a wide variety of alloys.

Colorimetric methods, such as those involving use of nitroso-R salt,[1] nitrosocresol,[2] thiocyanate[2] and other reagents,[3] although useful for determining trace amounts of cobalt in various materials, are not the most accurate for cobalt-bearing alloys unless the cobalt is first separated. One of the most common procedures for steels and alloys consists in preliminary treatment with zinc oxide to remove iron and some other interfering elements and then precipitation of the cobalt by 1-nitroso-2-naphthol. Reports on separations with

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zinc oxide differ considerably, and, if two separations are required, as frequently recommended, the procedure is lengthy. The cobalt complex is finally ignited to the oxide[4] and weighed as Co_3O_4. Since Co_3O_4 (a mixture of CoO and Co_2O_3) is of indefinite composition, in which the cobalt content can theoretically vary from 71 to 78·8 per cent., the method is subject to inherent errors.

The reduction of the cobalt oxides to metallic cobalt by heating in hydrogen has been recommended,[5] but this obviously adds to the length and complexity of the determination. An alternative step, equally difficult to carry out, consists in weighing the cobalt sulphate produced by treating the oxides with sulphuric acid.[5] A third method is electro-deposition of cobalt from an ammoniacal solution after the oxides have been re-dissolved. This step requires considerable time and care if complete deposition of the cobalt is to be obtained. Silver and copper must be absent or must be removed, since neither is completely removed by the treatment with zinc oxide[6] and both accompany cobalt in the precipitation by 1-nitroso-2-naphthol[7] and in the electro-deposition step.[8] A correction for sulphur is sometimes necessary.

A less common method of determining cobalt is by precipitation with potassium nitrite,[9] but the utility of this method is limited because of the number of interfering substances and the time required for complete precipitation.

Ion-exchange methods have been used for determining cobalt. Lewis and Straub[10] developed a routine determination involving final titration with ethylenediaminetetra-acetic acid; this was a modification of Hague, Maczkowske and Bright's method.[11] The detection of the end-point for cobalt by means of fluorescent indicators had been facilitated earlier by Wilkins.[12]

There are several volumetric methods for determining cobalt. One involves titration with potassium cyanide in ammoniacal citrate solution in the presence of silver nitrate and potassium iodide,[13] but copper and nickel also react with the cyanide. Elements such as niobium or tantalum may interfere by causing a turbidity that obscures the end-point, and high concentrations of chromium make the solution too dark for visual detection of the end-point. As at least one of the elements nickel, chromium, copper, tantalum and niobium is usually present in cobalt alloys, titration with cyanide is of limited use. A more widely used volumetric procedure for cobalt is potentiometric titration in ammoniacal citrate solution with potassium ferricyanide, as originally proposed by Dickens and Maassen.[14] The method is popular because it is rapid and almost as accurate as existing gravimetric procedures, but there are several objections to be overcome.[15] Among these are the irreversibility of the reaction between potassium ferricyanide and cobalt nitrate and the instability of certain electrodes in the strongly buffered solution. Moreover, certain elements, *e.g.*, tungsten, chromium or vanadium, may interfere, depending on the choice of electrodes. Most important is the fact that manganese reacts with potassium ferricyanide, although not quantitatively; this can lead to serious errors for alloys low in cobalt and containing higher amounts of manganese. The influence of high contents of titanium can also affect the procedure (personal communication from Mr. L. Silverman, North American Aviation Inc., Canoga Park, California, U.S.A.). However, several improvements, including the separation of manganese before titration, have been reported by Chirnside, Cluley and Proffitt.[16]

USE OF PHENYLTHIOHYDANTOIC ACID—

In 1922, Willard and Hall[17] used phenylthiohydantoic acid for quantitatively precipitating cobalt, and it is surprising that the value of this reagent has been subsequently almost unrecognised. Perhaps the price, commercial availability and purity of the reagent may have been a deterrent to its use. Another disadvantage is the tendency for iron and nickel to be co-precipitated with cobalt; with alloys containing large amounts of iron and nickel, this frequently produces a voluminous precipitate that is difficult to handle.

Willard and Hall were evidently seeking a specific reagent for forming an organo-metallic complex that could be weighed. Since this object was not attained and the precipitate could not be ignited to a constant form of oxide, their research was obviously concluded. They found, however, that, in slightly ammoniacal solution containing ammonium citrate, cobalt is quantitatively precipitated by phenylthiohydantoic acid; the brownish purple precipitate can be easily filtered and washed. Although several other elements are completely precipitated or partly co-precipitated, it is significant that, of the elements usually found in cobalt-bearing alloys, only iron, nickel and copper are in this class.

Iron, nickel and copper do not interfere in the potentiometric titration of cobalt with potassium ferricyanide, and manganese, which is the most troublesome element in this titration, is not precipitated by phenylthiohydantoic acid. It therefore appeared that a combination of these two methods might lead to an accurate procedure for determining cobalt in a wide variety of cobalt-bearing alloys. Experiments indicated that this was so, provided that certain initial difficulties could be overcome.

Experimental

Phenylthiohydantoic acid is readily soluble in hot ethanol, and a 4 per cent. w/v solution of the reagent was used in this work. Weights of sample were chosen to contain 10 to 40 mg of cobalt, since this range was most convenient for titration.

Conditions for precipitation—

Precipitation was carried out by adding the warm phenylthiohydantoic acid solution to a warm ammoniacal solution of the sample containing 10 g of ammonium citrate; the mixture was then allowed to cool. With alloys high in cobalt and low in iron, *e.g.*, S-816 alloys and National Bureau of Standards sample No. 167, purple - red precipitates, reasonably small and easy to filter and wash, were produced. Iron-based alloys containing 5 to 8 per cent. of cobalt, *e.g.*, high-speed steels, however, produced excessive amounts of a black precipitate. A 0·2-g portion of National Bureau of Standards sample No. 153, for example, produced a precipitate that half filled a 600-ml beaker and could not be contained in a 15-cm filter-paper. Analysis of this precipitate showed that 70 per cent. of the iron originally present in the 0·2-g sample had been co-precipitated with the cobalt. Although the form of the precipitate was conducive to washing, its large volume made efficient washing impossible, and to decrease the weight of sample in order to produce a precipitate that could be easily handled would reduce the cobalt content of the final solution to an extent such that the accuracy of the titration would be impaired.

The use of a complexing agent to prevent or decrease co-precipitation of iron was then tried. It was found that thioglycollic acid, when added just before the phenylthiohydantoic acid solution, formed a complex with the iron and markedly reduced its tendency to co-precipitation. For 0·2-g samples of iron-based alloys, 3 or 4 ml of thioglycollic acid were sufficient to maintain most of the iron in solution. Chemical determinations on British Chemical Standard and National Bureau of Standards samples, both with and without additions of thioglycollic acid, showed that the thioglycollic acid did not interfere with the precipitation of cobalt.

Independent of the amount of nickel present in the sample, small amounts of this metal are co-precipitated with the cobalt; this is not as troublesome as iron during filtration and in no way affects subsequent steps.

Precipitates produced by phenylthiohydantoic acid from solutions of pure cobalt tend to be finer than those produced from mixtures containing cobalt and iron. To ensure that all the precipitated cobalt is retained on the filter-paper when thioglycollic acid is used, the precipitate should be well digested with a little ashless filter-paper pulp before it is separated by filtration; the precipitate should be collected on a close-textured paper, such as Whatman No. 42 or its equivalent, and gentle suction can be applied.

The organo-cobalt precipitate and the filter-paper can easily be destroyed and the cobalt brought into soluble form by heating with a mixture of nitric and perchloric acids. When heated until fumes of perchloric acid are evolved, a point is reached at which evolution of fumes is rapid. This marks the beginning of the oxidation of final traces of organic matter, and heating should be discontinued to avoid mechanical loss of cobalt perchlorate solution. After dilution with water, the solution contains all the cobalt and only small amounts of iron and nickel (if these elements were present in the sample).

Most cobalt-bearing alloys can be decomposed with hydrochloric, nitric, perchloric or sulphuric acid or with appropriate mixtures of these acids. Heating to fumes with perchloric acid usually decomposes the carbides, and heating to fumes with orthophosphoric and sulphuric acids will dissolve alloys containing niobium, tantalum and tungsten (these elements remain in solution when citrate is added later and do not interfere with the precipitation of cobalt by phenylthiohydantoic acid).

CONDITIONS FOR TITRATION—

The success of the determination depends on the ease and accuracy of the titration step. Most of the work on the development of the proposed method was directed towards electrometrically determining the conditions for greatest sensitivity and accuracy of the titration end-point. Commercial apparatus suitable for titrating cobalt is of two main types; the first is primarily for potentiometric titrations and the second is essentially a pH meter. For this work, either a Fisher electrometric titrimeter (improved senior model) with an electron ray tube or a Beckman portable pH meter (model N) was used; other conditions being equal, the two instruments were similar in sensitivity. The Fisher titrimeter has a built-in stirring apparatus, but mechanically the Beckman pH meter was as convenient; it could be used with a magnetic stirrer, thereby leaving more space at the opening of the beaker for the two electrodes and the tip of the burette. Both instruments, however, were suitable. A glass - platinum electrode system was tried, but improved sensitivity was attained with a calomel - platinum system.

Sensitivity at the end-point was unaffected by the presence in the solution of up to 15 ml of perchloric acid, but tended to decrease as the amount of cobalt present was increased; there was a discernible difference between the sensitivities attained in presence of 20- and 50-mg amounts of cobalt. The presence in excess of between 40 and 120 ml of the ammonium citrate buffer and ammonia solutions used had little effect on sensitivity at the end-point.

The temperature of the solution during titration affects the accuracy of the end-point. The effect is almost constant between 70° and 85° F; above 85° F, the amount of cobaltous nitrate solution required to titrate a given amount of potassium ferricyanide solution decreases, and above 100° F errors are serious. Since the heat of reaction increases the temperature when the perchloric acid solution of the sample is added to the ammoniacal citrate buffer solution, the latter should be cooled to about 60° F before they are mixed. Cooling after mixing is not advisable, since the titration should be made immediately after the reagents have been mixed.

It is well known that bivalent cobalt is converted to the tervalent state only by the strongest oxidising agents and that the cobaltic ion is exceedingly unstable, being decomposed by water, even in the cold, with the evolution of hydrogen and the formation of cobaltous ions. In the presence of complexing agents, however, tervalent cobalt is stabilised. In ammoniacal solution, free ammonia serves as the complexing agent, with the formation of the hexammine cobaltic ion. This decreases the oxidation potential for the cobalt reaction so that it is sufficiently less than the ferrocyanide potential for the reaction to occur quantitatively. Ethylenediamine forms an even more stable complex with cobaltic ions, and in the presence of this reagent the potential is further decreased and cobaltous ions are more easily oxidised by ferricyanide. Diehl and Butler[18] substituted ethylenediamine for ammonium hydroxide with good results.

Tests showed that the addition of small amounts of ethylenediamine sharply increased the sensitivity at the end-point. In the presence of a few drops of ethylenediamine, a sharp break in the potential at the end-point occurred when a small drop of ferricyanide solution was added. When ethylenediamine was used, the end-point could be easily determined without having to plot a titration curve, as was sometimes necessary when titrating in the presence of ammonia only. Titrations of potassium ferricyanide solutions with standard cobaltous nitrate solution showed that the accuracy of the end-point was not impaired by the use of ethylenediamine.

The excess of ferricyanide in the sample is titrated with standard cobaltous nitrate solution in ammonium hydroxide - ammonium citrate buffer. Unless precautions are taken, fumes of ammonia react with the drop of cobaltous nitrate solution at the tip of the burette to form a precipitate, and some of the cobaltous nitrate may be oxidised by contact with air. Yardley[19] recommended that the solution be covered with a layer of light petroleum to exclude air and retain ammonia fumes. A more convenient method is to use a burette having a fine tip, which is submerged in the citrate buffer solution.

For measuring the standard potassium ferricyanide solution, a 25-ml burette accurate to 0·05 ml was convenient; for the standard cobaltous nitrate solution, a 10-ml microburette graduated in 0·05-ml divisions and having a fine tip should be used. During titrations, the solution should be stirred continuously, a magnetic stirrer being convenient. The rate of

stirring should be regulated so as not to cause splashing or to beat air into the solution. Livingston[20] has stated that the rotating field of a magnetic stirrer decreases the values shown by a pH meter by as much as 0·2 unit of pH. However, since the change in pH or potential, rather than the absolute value, indicates the end-point of the reaction, this error does not affect the accuracy of results. After the addition of ferricyanide solution, other reagents should be added immediately and the titration should proceed without delay, as we have found that ferricyanide may be partly reduced on prolonged contact with the ammoniacal citrate buffer.

Method

Range—

The procedure described below is effective for determining concentrations of cobalt from 3 per cent. upwards. As well as cobalt, the sample may contain aluminium, carbon, chromium, copper, iron, manganese, molybdenum, nickel, niobium, phosphorus, sulphur, tantalum, titanium, tungsten, vanadium and zirconium. The method is excellent for materials such as high-temperature alloys, high-speed steels and permanent-magnet alloys.

Apparatus—

Potentiometric-titration assembly—Fitted with platinum and calomel electrodes and with a suitable stirring apparatus, *e.g.*, a Fisher titrimeter with built-in stirrer or a Beckman pH meter (model N) in combination with a magnetic stirrer.

Burettes—One of capacity 10 ml and one of 25 ml, each graduated in 0·05-ml subdivisions.

Reagents—

Standard cobaltous nitrate solution—Dissolve 4 g of high-purity cobalt metal in 40 ml of nitric acid, boil for 5 minutes, and dilute to 2 litres with water.

1 ml ≡ approximately 0·002 g of cobalt.

Standard potassium ferricyanide solution—Dissolve 11·00 g of analytical-reagent grade potassium ferricyanide in water, and dilute to 1 litre. This solution is stable for at least 6 weeks if stored in the dark.

1 ml ≡ approximately 1 ml of the standard cobaltous nitrate solution.

Phenylthiohydantoic acid reagent solution—Dissolve 4 g of 4-phenyl-3-thiohydantoic acid in 100 ml of ethanol by heating at near the boiling-point of the ethanol.

Ammonium citrate buffer solution—Dissolve 750 g of citric acid in 1 litre of water, and add 1 litre of ammonia solution, sp.gr. 0·880, cooling the vessel in a stream of cold water during the addition.

Ammonium citrate wash solution—Dissolve 5 g of ammonium citrate in 1 litre of water, and add 5 ml of ammonia solution, sp.gr. 0·880, and 10 ml of the phenylthiohydantoic acid reagent solution.

Ethylenediamine, 95 to 100 per cent.

Thioglycollic acid, purified.

Ammonium citrate—Analytical-reagent grade.

Procedure—

Place 0·05 to 0·3 g of sample containing from 6 to 50 mg of cobalt in a 600-ml beaker, and decompose with a suitable mixture of hydrochloric and nitric acids (usually 3 volumes of hydrochloric acid and 1 volume of nitric acid); warm, if necessary, to initiate reaction. When the sample has dissolved, add 5 ml of 85 per cent. orthophosphoric acid, 20 ml of sulphuric acid diluted (1 + 1) and 5 ml of 70 per cent. perchloric acid. Evaporate until most of the perchloric acid has been removed or through the stage at which the chromium is oxidised to the red chromate and then partly reduced (excessive evaporation may cause formation of residues that are insoluble or soluble only with difficulty). Cool, add 25 ml of water, and boil for several minutes to dissolve the salts (see Note 1).

Dilute the solution to 150 ml with water, add 10 g of ammonium citrate, and stir until solution is complete. Add ammonia solution, sp.gr. 0·880, until the solution is slightly alkaline (pH 8; test with Hydrion or any other suitable indicator paper), and then add 10 ml in excess. Dilute to about 250 ml, and cool to about 35° C. Add 4 ml of thioglycollic acid (see Note 2), stir, set aside for 2 or 3 minutes, and then add a small amount of ashless filter-paper pulp. With stirring, add 35 ml of the phenylthiohydantoic acid reagent solution (see Note 3), heat

to boiling-point, stir vigorously until the precipitate coagulates, and allow to simmer for 5 minutes. Add 5 ml of the phenylthiohydantoic acid reagent solution, and digest at below boiling-point, if necessary, for a further 5 minutes.

Filter the hot mixture through a 15-cm Whatman No. 42 filter-paper containing a little ashless filter-paper pulp; use slight suction (see Note 4). Wash the precipitate five or six times with hot ammonium citrate wash solution, discard the filtrate and washings, and transfer the filter-paper and precipitate to the original beaker. Add 35 ml of nitric acid and 12 ml of 70 per cent. perchloric acid, immediately place the beaker on a hot-plate, and heat rapidly to destroy organic matter. Boil until the solution becomes clear and the perchloric acid becomes concentrated. At this stage, the action of the perchloric acid on the remaining organic matter causes rapid evolution of white fumes; when this occurs, remove the beaker from the hot-plate, and allow it to cool to room temperature.

Place 100 ml of ammonium citrate buffer solution in a 400-ml beaker, add 80 ml of ammonia solution, sp.gr. 0·880, and then add, from the 25-ml burette, sufficient standard potassium ferricyanide solution to react with the cobalt in the sample *plus* about 5 ml in excess (see Note 5); measure this volume to within 0·05 ml or less. Place the beaker on the titration assembly, insert the platinum and calomel electrodes, stir at a moderate rate, and add 6 drops of ethylenediamine to the solution.

Rinse the cover and sides of the 600-ml beaker containing the sample solution with a little water, pour the solution into the ferricyanide solution, again rinse the 600-ml beaker with a little water, and add the rinsings to the contents of the 400-ml beaker. Place the tip of the 10-ml burette in the sample solution, and titrate with the standard cobaltous nitrate solution, adding titrant slowly as the end-point is approached. Take the end-point as being when the last 0·05-ml portion of titrant added produces the greatest change in potential (or pH if a pH meter is used).

Similarly titrate exactly 10 ml of the standard potassium ferricyanide solution with the standard cobaltous nitrate solution, and determine the "cobaltous nitrate equivalent" of 1 ml of the ferricyanide solution. Determine the cobalt content of the standard cobaltous nitrate solution by analysing an alloy of known cobalt content, *e.g.*, National Bureau of Standards sample No. 153, as described above. Calculate the cobalt content of the sample from the expression—

$$\text{Cobalt content, \%} = \frac{[(C \times A) - B] \times 100\,D}{\text{Weight of sample, g}}$$

in which A is the amount of standard potassium ferricyanide solution used, B is the amount of standard cobaltous nitrate solution added, C is the "cobaltous nitrate equivalent" of 1 ml of the standard ferricyanide solution and D is the amount of cobalt equivalent to 1 ml of the standard cobaltous nitrate solution (calculated from the result of the determination on the sample of known cobalt content).

NOTES—

1. This treatment should give a clear solution, even for samples containing niobium, tantalum or tungsten, but some alloys of high carbon content may leave an insoluble black residue. An alternative procedure is to add 15 ml of 70 per cent. perchloric acid after the decomposition with hydrochloric and nitric acids and then to evaporate until dense fumes are evolved. Cool slightly, add 50 ml of water, boil for a few minutes, and filter through a Whatman No. 40 filter-paper. Wash the residue with hot water, add the washings to the filtrate, and ignite the filter-paper and insoluble matter in a platinum crucible. Fuse the residue with a little sodium pyrosulphate, allow the melt to cool, leach with sulphuric acid, and filter the solution. Combine the filtrate with the main filtrate, and continue as described above, beginning at "Dilute the solution to 150 ml with water. . . ."
2. The addition of thioglycollic acid can be omitted if the sample is low in iron. Samples having high contents of iron produce precipitates too bulky to handle unless thioglycollic acid is used.
3. Theoretically, 1 ml of the phenylthiohydantoic acid reagent solution will precipitate 5·6 mg of cobalt; an excess of three times the theoretical amount is used. Forty millilitres of the reagent solution are sufficient to precipitate 224 mg of cobalt, assuming that no other elements that will react, *e.g.*, antimony, bismuth, cadmium, copper, lead, mercury, selenium and silver, are present.
4. If no thioglycollic acid was added, the precipitate can be collected on a 15-cm Whatman No. 41H filter-paper, without suction. As the filtrate cools, it will become turbid owing to precipitation of the excess of phenylthiohydantoic acid.
5. An excess of potassium ferricyanide must be present at the beginning of the titration, as the reaction is not reversible. If the approximate cobalt content of the sample is not known, carefully watch the potential (or pH) as the sample solution is poured into the ferricyanide solution. With a little experience, the operator can tell if sufficient ferricyanide is present and can add more, if necessary, before adding the rest of the sample solution.

Results

The accuracy of the proposed method was checked by determining the cobalt contents of four standard alloys, the nominal compositions of which are shown in Table I; the results are summarised in Table II. The standard cobaltous nitrate solution used in these determinations was made from cobalt sponge specially prepared as a spectrographic standard. This sponge was assumed to contain 100 per cent. of cobalt, and no factors were used in calculating the results. (Spectrographic analysis of the sponge showed that it contained less than 0·005 per cent. of metallic impurities.)

Table I

Nominal compositions of standard alloys

Element	Concentration of element in— sample No. N.B.S. 153, %	sample No. N.B.S. 167, %	sample No. B.C.S. 233, %	sample No. B.C.S. 241, %
Cobalt	8·45	42·90	23·72	5·84
Aluminium	—	0·025	6·98	—
Carbon	0·864	0·38	—	0·83
Chromium	4·14	20·00	—	5·12
Copper	0·099	0·03	5·09	0·15
Iron	Balance	2·13	51·15	Balance
Manganese	0·299	1·64	0·235	0·23
Molybdenum	8·38	3·90	—	0·54
Nickel	0·107	20·65	11·22	0·19
Niobium	—	3·15	—	—
Nitrogen	—	0·07	—	—
Phosphorus	0·025	0·010	—	0·025
Silicon	0·187	0·44	0·57	0·22
Sulphur	0·008	0·007	—	0·035
Tantalum	—	0·08	—	—
Tin	—	—	—	0·035
Titanium	—	0·01	0·79	—
Tungsten	1·58	4·50	—	20·38
Vanadium	2·04	0·01	—	1·54

Table II

Cobalt found in standard alloys

Sample No.	Cobalt present, %	Average cobalt content found, %	Number of determinations	Standard deviation, %
N.B.S. 153	8·45	8·49	19	0·17
N.B.S. 167	42·90	42·95	18	0·33
B.C.S. 233	23·72	23·84	19	0·18
B.C.S. 241	5·84	6·01	19	0·075

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Rapid Complexometric Determination of Phosphorus in Coal

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A complexometric method for determining phosphorus in coal has been developed. Phosphorus is extracted from coal ash by treatment with perchloric acid and is then precipitated as ammonium magnesium phosphate. Magnesium in the precipitate is subsequently determined by titration with ethylenediaminetetra-acetic acid. The method is simple and rapid. The maximum mean deviation of replicate determinations was 0·003 per cent., but it was generally less than 0·0015 per cent. The accuracy is similar to that of the British Standard method. By using the proposed procedure, one worker can analyse five samples per day.

Recent trends[1 to 9] in the development of methods for determining phosphorus in coal, coke and their ash are characterised by the use of mixed mineral acids to extract phosphorus from the parent material, with the simultaneous removal of silica. In almost all these methods, subsequent determination of phosphorus in the silica-free extract involves formation of molybdophosphate, which is then determined spectrophotometrically or polarographically. These procedures have advantages over the British Standard method,[10] *viz.*, the time for a determination is considerably shorter and the use of platinum ware and hydrofluoric acid is avoided.

This paper describes a method for extracting phosphorus from coal ash by treatment with perchloric acid. Phosphorus is precipitated from the extract as ammonium magnesium phosphate after interfering metal ions have been screened by the use of a mixture of masking agents, *e.g.*, ethylenediaminetetra-acetic acid (EDTA) or nitrilotriacetic acid (NTA), citric acid, triethanolamine and ammonium tartrate; it has been found that EDTA can be replaced by NTA. The phosphorus content of the ash is then found indirectly by determining the magnesium in the precipitate complexometrically.

Method

Reagents—

All reagents should be of recognised analytical grade.

Perchloric acid, 60 to 72 per cent.

EDTA solution, approximately 0·1 M.

NTA solution, approximately 0·1 M—Prepare as described by Schwarzenbach.[11]

Citric acid.

Ammonium tartrate.

Triethanolamine solution—Mix equal volumes of triethanolamine and water. Store this solution in a polythene bottle.

Magnesium chloride - ammonium chloride reagent solution—Dissolve 50 g of magnesium chloride hexahydrate and 100 g of ammonium chloride in 1 litre of water, add a few drops of ammonia solution, and set aside overnight. Filter, if necessary, and acidify with 4 ml of hydrochloric acid, sp.gr. 1·18.

Buffer solution, pH 10—Dissolve 80 g of ammonium nitrate in water, add 350 ml of ammonia solution, sp.gr. 0·90, and dilute to 1 litre. Store this solution in a polythene bottle.

Standard magnesium sulphate solution—Dissolve about 2·5 g of magnesium sulphate heptahydrate in 1 litre of water. Standardise by titration against 0·01 M EDTA, with Eriochrome black T as indicator.

Standard EDTA solutions, 0·01 and 0·005 M.

Sodium hydroxide solution, 30 per cent. w/v—Store in a polythene bottle.

Eriochrome black T indicator solution—Dissolve 0·2 g of the indicator in a mixture of 25 ml of absolute ethanol and 75 ml of triethanolamine.

PROCEDURE—

Prepare coal ash by the British Standard method,[10] and accurately weigh 200 mg of dry finely ground ash (500 mg for samples containing less than 0·2 per cent. of phosphorus) into a Pyrex-glass 250-ml conical beaker. Digest on a hot-plate with 10 to 15 ml of perchloric acid for 15 to 20 minutes, with occasional swirling, and add more perchloric acid if necessary. Heat until dense fumes are evolved, cool, and dilute to 50 ml with hot water, washing down the sides of the beaker. Allow to settle for 5 minutes, filter through a Whatman No. 42 filter-paper covered with a layer of filter-paper pulp, and collect the filtrate in a Pyrex-glass 600-ml beaker. Wash the residue five or six times with hot water, and add the washings to the filtrate; the volume of the combined filtrate and washings should not exceed 200 ml. To the cooled filtrate add about 0·2 g of ammonium tartrate, about 0·1 g of powdered citric acid, 10 ml of triethanolamine solution and then 10 ml of 0·1 M EDTA or NTA. Add 20 ml of cold magnesium chloride - ammonium chloride reagent solution, 1 drop of methyl red indicator solution and then concentrated ammonia solution, dropwise, until the colour of the indicator changes. Stir vigorously until precipitation of ammonium magnesium phosphate begins, add 5 ml of concentrated ammonia solution, and stir for a further 5 minutes. Set aside in a cold place for 2 hours, and separate the precipitate on a Whatman No. 42 filter-paper. Wash the beaker and the residue with M ammonia solution until a 10-ml portion of the washings turns blue when 1 drop each of Eriochrome black T indicator solution and 0·01 M EDTA are added.

Pierce a hole in the filter-paper, and transfer the precipitate with a jet of hot water to the original beaker. Wash the filter-paper twice with hot 3 N hydrochloric acid and then a few times with hot water, collect the washings in the beaker, and dissolve all the precipitate (by warming, if necessary). Neutralise the solution with 30 per cent. sodium hydroxide solution (use a small piece of methyl red paper as indicator), add 10 ml of buffer solution and a few drops of Eriochrome black T indicator solution, and immediately titrate with 0·01 or 0·005 M EDTA until the colour changes from wine-red to pure blue.

1 ml of 0·01 M EDTA ≡ 0·31 mg of phosphorus.

If the amount of precipitated ammonium magnesium phosphate is large, dissolve the washed precipitate in hydrochloric acid, and add 25 ml of 0·01 M EDTA by pipette. Neutralise as described above, adjust the pH to 10, and titrate the excess of EDTA against standard magnesium sulphate solution, with Eriochrome black T as indicator; the colour changes from blue to wine-red at the end-point.

DISCUSSION OF THE METHOD

To four solutions containing the ions Fe^{3+}, Al^{3+}, Ti^{4+}, Ca^{2+}, Mg^{2+}, Na^{+}, K^{+} and SO_4^{2-} were added accurately weighed amounts of anhydrous disodium hydrogen orthophosphate that had been dried at 120° C; the solutions were then diluted to known volumes. (The amounts of metal ions in these solutions were representative of the relative proportions normally found in ash from Indian coal.) The phosphorus contents of these solutions were determined in triplicate by the proposed procedure, EDTA being used as masking agent in some of the determinations and NTA in the others. The results are shown in Table I, from which it can be seen that recovery of phosphorus was 99·5 to 100 per cent.

Fifteen Indian coals having different contents of phosphorus were analysed by the proposed and British Standard[10] procedures; the results (see Table II) were in close agreement. The phosphorus content of each sample was generally determined six times. The agreement between the results for each sample and between the mean values for the two procedures

is indicative of the reproducibility and accuracy of the proposed method. As before, the use of NTA in place of EDTA as masking agent in some of these determinations produced no appreciable difference in the results. Hence, either of these masking agents can be used.

TABLE I

RECOVERY OF PHOSPHORUS FROM PREPARED SOLUTIONS

Each solution also contained the ions Fe^{3+}, Al^{3+}, Ti^{4+}, Ca^{2+}, Mg^{2+}, Na^{+}, K^{+} and SO_4^{2-}

Solution No.	Phosphorus added, mg	Phosphorus found, mg	Average recovery, %
1	3·110	3·115, 3·131,* 3·038 (mean 3·095)	99·84
2	1·550	1·558,* 1·550, 1·5345 (mean 1·550)	100·0
3	0·620	0·614,* 0·617, 0·620* (mean 0·617)	99·52
4	0·465	0·465, 0·462,* 0·468 (mean 0·465)	100·0

* NTA used as masking agent.

TABLE II

COMPARISON OF RESULTS FOR COALS BY PROPOSED AND BRITISH STANDARD METHODS

Determinations by the British Standard method were made by a separate group of analysts

Sample No.	Phosphorus content found by proposed method, %	Mean phosphorus content found by— proposed method, %	Mean phosphorus content found by— British Standard method, %	Mean deviation of results by proposed method, %
1	0·459, 0·465, 0·466, 0·470,* 0·468,* 0·463	0·465	0·458	0·0030
2	0·297, 0·297, 0·297, 0·297,* 0·297,* 0·265	0·297	0·297	0·0003
3	0·269, 0·269,* 0·269, 0·269, 0·267,* 0·269,	0·269	0·273	0·0003
4	0·101, 0·100, 0·100,* 0·098,* 0·100	0·100	0·100	0·0006
5	0·177, 0·182, 0·182,* 0·182, 0·177*	0·180	0·178	0·0024
6	0·357,* 0·357,* 0·357, 0·362, 0·357, 0·357	0·358	0·349	0·0015
7	0·228, 0·231, 0·228,* 0·231,* 0·228*	0·229	0·232	0·0014
8	0·117, 0·114, 0·116, 0·119, 0·117	0·117	0·119	0·0012
9	0·108, 0·111, 0·110, 0·108, 0·108, 0·108*	0·109	0·110	0·0012
10	0·215, 0·214, 0·216, 0·214, 0·214, 0·214	0·214	0·213	0·0005
11	0·091, 0·091, 0·093, 0·091, 0·093, 0·093	0·092	0·098	0·0010
12	0·074, 0·074, 0·073, 0·078,* 0·074,* 0·077	0·074	0·077	0·0013
13	0·008, 0·009, 0·008, 0·008, 0·008, 0·007*	0·008	0·007	0·0003
14	0·041, 0·042, 0·040, 0·044,* 0·044*	0·042	0·041	0·0014
15	0·385,* 0·385, 0·388,* 0·388, 0·393	0·388	0·396	0·0022

* NTA used as masking agent.

After solution of the precipitated ammonium magnesium phosphate, buffer solution is added to adjust the pH to 10 before the final titration with EDTA solution. If the amount of precipitate is large (equivalent to more than 10 mg of P_2O_5), the ammonium magnesium phosphate may be re-precipitated during the titration, so giving low results. In such circumstances, the back-titration procedure described above is adopted. However, when the

amount of precipitate is small, the tendency of ammonium magnesium phosphate to form supersaturated solutions presents no difficulty and direct titration is permissible.

The proposed method is simple, accurate and rapid. It obviates the use of costly platinum ware, which is not generally available for simultaneous batch analyses. The main advantage of this procedure over the British Standard method consists in the avoidance of the strict adherence to specified experimental conditions necessary to produce a precipitate of molybdophosphate having the correct composition and in the subsequent filtration and washing of the rather unstable precipitate.

Finally, the proposed procedure, although primarily developed for determining phosphorus in coal and coke, could well be extended to such materials as blast-furnace slag, iron ore, boiler deposits and oil shales.

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Notes

AN ION-EXCHANGE SEPARATION AND POLAROGRAPHIC DETERMINATION OF SMALL AMOUNTS OF LEAD AND ZINC IN THE PRESENCE OF COBALT

KRAUS and Moore's anion-exchange studies[1] indicated a practical approach to the separation of zinc from an excess of the transition elements. These workers showed that, in various concentrations of hydrochloric acid, the anionic complex chlorides of zinc and the members of the first transition group of elements differed in ion-exchange behaviour to an extent such that they could readily be separated on a strongly basic ion-exchange resin (Dowex 1). A graph of elution constant against concentration of acid indicated the feasibility of separating not only zinc from the transition group, but also the individual members of the group, including cuprous copper, from each other.

This principle has been used in these laboratories, and a method was developed for the rapid separation of trace amounts of nickel from copper, cobalt and iron.[2] Miller and Hunter[3,4] used a similar technique, with moderate success, to separate 5 to 50 mg of zinc from 100 mg of metal ions and noted the similarity between the retentive characteristics of the anionic chlorides of lead, indium and zinc. Experiments in these laboratories on the quantitative aspect of the anion-exchange separation of zinc from solutions of commercial cobalt metal indicated that lead was tenaciously adsorbed by the resin De-Acidite FF in the presence of hydrochloric acid of molarity greater than 0·005. Further, it was shown that both lead and zinc could be eluted by a solution having an extremely low concentration of acid and could be readily determined by a polarographic procedure based on Semerano and Gagliardo's method.[5]

Experimental

To study the efficiency with which lead and zinc were separated from relatively large amounts of cobalt, copper, iron, nickel and manganese, six solutions containing 1 g of each of these metals

in 50 ml of 2 N hydrochloric acid were prepared. To each solution were added standard solutions to cover the range 0 to 1·0 mg each of lead and zinc; care was taken to ensure that the total volume added did not exceed 2·0 ml, thereby maintaining the concentration of acid in the final solution at nearly 2 N. Each solution was allowed to percolate at 75 to 100 drops per minute through an ion-exchange column prepared as described under "Method." When the level of solution had fallen to the top of the resin, 50 ml of 2 N hydrochloric acid were added and allowed to run through. This addition of acid was repeated until an almost colourless effluent indicated that all the nickel and most of the cobalt had been eluted. The complete removal of nickel, cobalt, copper, iron and manganese from the column was then accomplished by the passage of four successive 50-ml portions of 0·5 N hydrochloric acid, and, finally, lead and zinc were eluted with 100 ml of 0·005 N hydrochloric acid. The fraction of effluent containing lead and zinc was evaporated to dryness, and the residue was dissolved in 40 ml of base electrolyte solution. Buffer solution (5 ml) and gelatin solution (0·5 ml) were added, the mixture was diluted to exactly 50 ml, and a polarogram was recorded for lead and zinc as described under "Preparation of Standard Graphs." The diffusion currents were measured and referred to graphs prepared from results for standard solutions of lead and zinc. The recoveries of lead and zinc are shown in Table I.

TABLE I

RECOVERIES OF ADDED LEAD AND ZINC FROM PREPARED SOLUTIONS

Each solution contained 1 g each of cobalt, copper, iron, nickel and manganese

Lead added, mg	Zinc added, mg	Lead found, mg	Zinc found, mg
0	0	Nil	Nil
0·2	0·2	0·19	0·18
0·4	0·4	0·38	0·38
0·6	0·6	0·58	0·57
0·8	0·8	0·76	0·76
1·0	1·0	0·94	0·96

METHOD

REAGENTS—

Unless otherwise stated, all materials should be of recognised analytical grade.

Hydrochloric acid, 2, 0·5 *and* 0·005 N.

Base electrolyte solution—Dissolve 250 g of sodium chloride in 900 ml of distilled water, add 50 ml of 2 N hydrochloric acid, and dilute to 1 litre.

Buffer solution—Dissolve 385 g of ammonium acetate in 500 ml of distilled water, add 286 ml of glacial acetic acid, and dilute to 1 litre.

Gelatin solution—Dissolve 1·0 g of pure gelatin in 90 ml of hot distilled water, and dilute to 100 ml.

Wash solution—Dilute 10 ml of concentrated hydrochloric acid to 900 ml with distilled water, add 10 ml of water saturated with sulphur dioxide, and dilute to 1 litre.

Standard lead solution, 0·10 mg per ml—Dissolve 0·1077 g of Specpure lead oxide (catalogue No. JM564) in dilute nitric acid, evaporate to incipient dryness, and add 10 ml of concentrated hydrochloric acid. Evaporate to about 2 ml, and dilute to 1 litre.

Standard zinc solution, 0·10 mg per ml—Dissolve 0·1000 g of HS zinc rod (obtainable from Johnson, Matthey and Co. Ltd., catalogue No. JM150) in dilute hydrochloric acid, and dilute to 1 litre.

PREPARATION OF ANION-EXCHANGE COLUMN—

Gently wet-grind about 50 g of De-Acidite FF or a similar strongly basic anion-exchange resin until it passes through a 50-mesh sieve, but is retained by a 100-mesh sieve, without too much loss as "fines." Carefully place a loosely packed plug of glass-wool in the constriction of a 100-ml burette, and close the tap. Pour in the sifted resin, as a slurry in 1 per cent. v/v hydrochloric acid, until the settled bed occupies about one-third of the burette. Allow the acid to percolate through the column until the level is just above the top of the resin. Wash the column successively with 300 ml of the wash solution, 1 litre of distilled water and 300 ml of 2 N hydrochloric acid. Eluting agent will pass through such a column at 60 to 100 drops per minute.

PREPARATION OF STANDARD GRAPHS—

The ranges of lead and zinc concentrations to be covered by the calibration graphs will depend on the type and amount of sample to be analysed. The procedure described below is suitable for up to 1 mg of both metals.

Place in each of six 50-ml calibrated flasks 0, 0·2, 0·4, 0·6, 0·8 and 1·0 ml of standard lead and zinc solutions. To the contents of each flask add 40 ml of base electrolyte solution, 5 ml of buffer solution and 0·5 ml of gelatin solution, mix thoroughly by shaking, and dilute to the mark. Transfer each solution to a polarographic cell containing a static pool of mercury as anode. Thermostatically control the temperature of the cell at about 5° ± 0·5° C above the average room temperature. With a mercury drop rate of about 3 seconds, record a polarogram over the ranges −0·3 to −0·7 volt for lead and −1·0 to −1·45 volts for zinc (use an instrument sensitivity such that scale coverage for the 1-mg standards is about 90 per cent.). Measure the heights of the waves for lead and zinc (half-wave potentials −0·5 and −1·3 volts, respectively), and plot graphs of wave height against concentration; the graphs are linear.

APPLICATIONS OF THE METHOD

I have found the proposed method to be invaluable for determining trace amounts of lead and zinc in cobalt metal, cobalt and copper concentrates rich in copper and iron and in leach and electrolytic-process solutions containing various concentrations of sulphuric acid. For concentrates, the sample was decomposed with a mixutre of nitric and perchloric acids and then evaporated twice with hydrofluoric acid before separation by ion exchange.

Although the proposed method has been applied only to certain products of cobalt refining, I consider that the chemistry of the process would permit the method to be applied to the analysis of alloys, such as brasses and bronzes, and of all samples in which lead and zinc have to be separated from copper and the metals of the first transition series. In the analysis of nickel, copper and cobalt electroplating solutions it might be particularly useful; it might also provide an alternative to Haim and Barnes's method[6] for steels, which I have sometimes found difficult to apply.

I thank the Anglo American Corporation of South Africa Ltd., Salisbury, Southern Rhodesia, for permission to publish this Note and Mr. H. W. B. Collier for carrying out some of the routine determinations.

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THE FLUORIDE CONTENT OF GELATIN

INFORMATION on the fluoride content of gelatin is limited; Lockwood reported "nil" in one sample,[1] Stuart, Dahle and Frey reported 5 to 10 p.p.m. in six samples,[2] Moisiev reported 1·25 p.p.m.,[3] and Pektor and Navrátil found 20 to 200 p.p.m.[4] As part of a programme to determine the heavy-metal and fluoride contents of a number of foods and food chemicals,[5,6,7] several samples of gelatin were analysed and found to contain appreciable amounts of fluoride. In view of these results it was decided to extend this study. The method used for the determination is described below.

A 5-g sample of gelatin was placed in a platinum dish, and 25 ml of a 1 N suspension of lime and a few drops of olive oil were added. The sample was dried and then ignited at 600° C. The ash was dissolved in perchloric acid, and the fluoride was distilled as fluorosilicic acid.[8] The fluoride content of an aliquot of the distillate was determined by the thorium - thoron colorimetric procedure.[9]

Results

The fluoride contents of eighty-two samples of gelatin from ten countries are summarised in Table I, and the distribution of results is shown in Table II; the median value for fluoride content was 6·5 p.p.m. Of the samples examined, fifty-nine contained 10 p.p.m. or less of fluoride and seventy-four contained less than 25 p.p.m. Eight of the samples, which were processed with water containing 1 p.p.m. of added fluoride, contained 29 to 34 p.p.m. of fluoride.

Table I

Fluoride contents of samples of gelatin

Country of origin	Number of suppliers	Number of samples	Fluoride content		
			Minimum, p.p.m.	Maximum, p.p.m.	Average, p.p.m.
Argentina	1	4	12	19	16
Australia	1	5	3	10	5
Belgium	1	5	5	6	5
Canada	1	5	3	6	4
Denmark	1	2	7	9	8
England	4	21	2	7	4
France	2	6	2	9	5
Germany	1	2	1	1	1
Netherlands	1	4	4	7	5
U.S.A. ..	7	20	4	25	11
	2	8*	29	34	31

* Known to have been produced with use of water containing 1 p.p.m. of added fluoride.

Table II

Distribution of fluoride contents

Fluoride content, p.p.m.	Number of samples
0 to 1·5	4
1·6 to 2·5	6
2·6 to 3·5	6
3·6 to 5	20
5·1 to 7·5	19
7·6 to 10	4
10·1 to 16	11
16·1 to 25	4
25·1 to 34	8*

* Produced with use of water containing 1 p.p.m. of added fluoride.

Since gelatin is prepared by evaporating an extract solution (3 to 5 per cent.), any fluoride present in the water supply will be concentrated proportionately. If water containing 1 p.p.m. of fluoride is used, the level of fluoride in the finished gelatin from this source alone would be 20 to 30 p.p.m. Further, lime and other chemicals used in the production of gelatin might be expected to contribute some fluoride to the finished product. Samples containing less than 10 p.p.m. of fluoride probably show the minimal level to be expected in gelatin, whereas gelatins containing more than 10 p.p.m. of fluoride may have been produced by using water containing traces of natural or added fluoride or may have been derived from skins and hides treated with lime containing excessive amounts of fluoride.[4]

I thank Mr. J. S. Frayne, who carried out many of the determinations, for technical assistance.

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THIOFORMANILIDE AS A PRECIPITANT FOR METAL SULPHIDES: A QUALITATIVE APPROACH

THIOFORMANILIDE, described[1] as pale-yellow needles, melting-point 130° C, can be easily prepared by Hoffmann's procedure.[2] When heated, it decomposes into hydrogen sulphide and phenyl isocyanide; it also decomposes when warmed in solution to liberate hydrogen sulphide. Preliminary studies with a solution of thioformanilide in dioxan or ethanol indicated that the presence of acid facilitated liberation of hydrogen sulphide, even in the cold. The reagent therefore appeared to be suitable for precipitating metal sulphides from solutions of salts. We have investigated the qualitative use of thioformanilide for this purpose.

EXPERIMENTAL

Solutions containing about 1 per cent. of the metals tested were prepared in water or hydrochloric acid. These solutions were treated with a few millilitres of a 3 to 4 per cent. solution of thioformanilide in dioxan, and the effects were noted in neutral, acid (acidified with 2 N acetic or 2 N hydrochloric acid) and ammoniacal (2 N ammonia solution) media, both with and without heating. Almost equivalent amounts of reagent and cation were necessary for complete precipitation, but an excess of the reagent was always used, 2 to 3 ml of the thioformanilide solution being added to 10 ml of the 1 per cent. solution of the cation.

The initial precipitates were always yellowish white to deep yellow and were definitely not sulphides. However, except for copper, antimony and mercury^{I}, they changed to sulphides when set aside for 1 or 2 minutes or after slight warming. If the mixture was boiled after the reagent had been added, the sulphide was always obtained almost instantaneously. The compound obtained in the cold from ammoniacal, acid or neutral solutions of copper was a yellow emulsion, which changed to sulphide after the mixture had been boiled for about 5 minutes. Antimony in neutral or acid solution gave a white precipitate in the cold; this changed to the orange sulphide when warmed slightly. Mercury^{I} in neutral solution gave a yellow precipitate, which yielded the black sulphide when boiled.

DISCUSSION OF RESULTS

The conditions under which many sulphides were precipitated are summarised in Table I. These results clearly show that thioformanilide can serve as a sulphide precipitant for many metallic ions under suitable conditions. However, the formation of a yellowish white or yellow compound before precipitation of the sulphide indicates prior formation of a metal chelate, which, when set aside, stirred or boiled, breaks down to the metal sulphide and the hydrolysis products of the reagent.

TABLE I

CONDITIONS FOR PRECIPITATION OF SULPHIDES BY THIOFORMANILIDE

Ions precipitated in the cold*	Ions precipitated on warming
Solution acidified with hydrochloric acid—	
Hg^{2+}, Bi^{3+}, As^{3+}	Sb^{3+}, Cu^{2+}
Solution acidified with acetic acid—	
Ag^{+}, Pb^{2+}, Zn^{2+}	Cd^{2+}
Solution ammoniacal—	
Pb^{2+}, Sn^{2+}, Zn^{2+}, Mn^{2+}	Cu^{2+}, Ni^{2+}, Co^{2+}
Solution neutral—	
Ag^{+}, Hg^{2+}, Hg^{+}, Bi^{3+}, Zn^{2+}, Mn^{2+}, Cu^{2+}, Cd^{2+}	Sb^{3+}, Sn^{2+}, Ni^{2+}, Co^{2+}

* At room temperature, about 28° C.

Thioformanilide exists in the tautomeric forms $C_6H_5{\cdot}NH{\cdot}CH{:}S$ (**I**) and $C_6H_5{\cdot}N{:}CH{\cdot}SH$ (**II**). Form **II** is ideal for chelate formation, since it has a donating nitrogen atom and an acidic —SH group. Form **I** has an acidic —NH group and a donating sulphur atom, but the —NH group is only weakly acidic and the possibility of chelation with it is remote. The exact nature of the intermediate chelate cannot be ascertained until systematic work has been carried out, but we have established the ability of thioformanilide to precipitate sulphides.

The reagent can be dissolved in ethanol or dioxan for homogeneous precipitation and such a solution can be stored in a tightly stoppered bottle for 1 to 2 weeks without decomposition. The

time required for precipitating sulphides by this reagent is much less than that needed when hydrogen sulphide is used. Thioformanilide therefore appears to be a suitable precipitant for sulphides, even when compared with thioacetamide; the latter reagent completely precipitates most cations, but not those of cadmium, and tin^{IV} and lead.[3]

Work on the quantitative use of thioformanilide is in progress.

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Book Reviews

ANALYTICAL APPLICATIONS OF DIAMINO-ETHANE-TETRA-ACETIC ACID. By T. S. WEST and A. S. SYKES. Second Edition. Pp. 127. Poole, Dorset: The British Drug Houses Ltd. 1960. Price 4s. 6d.

Every opportunity seems to have been taken to correct and improve structural formulae criticised in a review of the first edition (*Analyst*, 1959, **84**, 673). A few typographical errors still remain (p. 17, a co-ordinate bond in place of an ordinary covalency; p. 19, square brackets omitted; p. 30, two bonds missing), and doubt may be cast on the formula given for the complex of murexide with calcium ions (p. 23). The (correct) formula for the complex of Ca^{2+} with NTA (p. 93) should remove any misapprehension that might have arisen from the statement "there are only three carbonyl oxygens and one basic nitrogen to react . . .," but the absurdity on p. 19 ("Since the concentration of the insoluble precipitate may be neglected . . .") has been carried over from the original.

Entirely new material is developed in an Appendix of 16 pages, which refers to work on primary standards and the theory of end-point detection and to new indicators, such as Methyl Thymol Blue, Glycine Thymol Blue, Naphthol Violet, Alizarin Complexone, Acid Alizarin Black, Fast Sulphon Black, Naphthyl Azoxine and Fast Grey RA. A good new feature is the designation of commercial dyestuffs by their numbers in the Colour Index. Reference is also made to enthalpy titrations, the use of mercury electrodes and to other physico-chemical procedures. Recent work on masking agents and the applications of amalgams in complexometric titrations is reviewed, together with a brief account of miscellaneous applications of EDTA and some of the newer complexones.

A select bibliography of books on EDTA and 55 new references complete the second edition of a compact and valuable little monograph.

H. IRVING

BRITISH PHARMACOPOEIA 1958: ADDENDUM 1960. Pp. xxii + 83. London: The Pharmaceutical Press for the General Medical Council. 1960. Price 30s.

This addendum consolidates the previously published amendments to the 1958 edition of the British Pharmacopoeia and includes forty-one new amendments and forty-nine new monographs. Fifteen of these deal with new drugs and the remainder with new salts or derivatives of compounds to be found in the 1958 or earlier editions. Although most of the newcomers are well-established synthetic drugs, the antibiotic novobiocin, the hormone corticotrophin (ACTH) and liothryonine sodium (L-tri-iodothyronine sodium) appear for the first time.

Important changes have been made in the assay of vitamin A, in which chromatographic separation is advised in certain cases, but it is not stated how this is done.

A revised method is described for the determination of molecular size in dextran injection, and a method for the bioassay of corticotrophin is included.

NORMAN EVERS

FLAME PHOTOMETRY. By JOHN A. DEAN. Pp. viii + 354. New York, Toronto and London: McGraw-Hill Book Co. Inc. 1960. Price 89s.

It should be stated at once that this is a most important book written by an author who is an acknowledged authority in the field of flame photometry. This technique has become a valuable and popular method of analysis, but has for too long been used in many laboratories on an entirely empirical basis. This book should do much to create a better understanding of the technique. The book is divided into four sections. In the first, "Theoretical Principles," the source unit is dealt with at some length, from atomisation to the characteristics of flames and the excitation of spectra; a special chapter is given to the use of organic solvents. The second section is devoted to instrumentation, interferences and methods of evaluation. In the third part, the elements to which the technique can be applied are treated systematically at some length, and the fourth section has three chapters on applications to clinical problems, soils and plant materials and cement and glass. No attempt has been made to include a full bibliography, because such compilations are available elsewhere, but 780 references are given and the fact that they were selected increases the value of this section.

The author shows a distinct preference for the combined atomiser - burner source unit, which is almost standard equipment in the U.S.A., and he describes it as a "total combustion burner." I am a little unhappy with this name; it may be truly descriptive when samples in organic solvents are being examined because evaporation of the droplets is usually complete and the whole of the sprayed sample is then available in the flame. In fact, the solvent may comprise the major proportion of the fuel. However, when aqueous solutions are used the larger drops go right through the flame and there is nothing like a total combustion of the sprayed sample.

However, this is a very minor criticism. The book is excellently written, contains nothing that can be regarded as superfluous and is a "must" for anyone practising or thinking of using flame-photometric methods. It is likely to be considered a standard work for some time to come.

L. BREALEY

TINPLATE TESTING: CHEMICAL AND PHYSICAL METHODS. By W. E. HOARE, D.Sc., F.I.M., and S. C. BRITTON, M.A., F.R.I.C., F.I.M. Pp. 55. Greenford, Middlesex: Tin Research Institute. 1960. Gratis.

A collection of test methods for tinplate, compiled by two senior members of the Tin Research Institute, will be welcomed by everyone concerned with the manufacture or use of tinplate. As a companion to the Tinplate Handbook, published a few years ago by one of the authors, it is designed to assemble a number of test procedures capable of assessing those properties of tinplate that influence its performance in manufacturing processes and in service. Methods for measuring mechanical properties of tinplate have been described previously, and this publication therefore confines itself deliberately to the qualities of tinplate surfaces.

The selected tests are discussed under nine headings, each dealing with a particular property of tinplate. The principles of the procedures and the significance of the results that can be obtained are described in the first half of the booklet. Detailed instructions for carrying out the examinations are given in a series of fourteen appendices, which form the second half of the publication. These instructions are written with admirable clarity, are well illustrated and should enable the reader to carry out the tests without difficulty.

Two classes of procedures are discussed in the publication: "Objective Tests" deal with well defined quantities, such as weights of tin-coating and amounts of tin oxide associated with the materials under test. The second group, "Subjective or Performance Tests," attempts to describe properties that at present cannot be assessed by clearly defined chemical or physical factors. It is an indication of the state of our knowledge that the authors have felt obliged to rely on this type of testing procedure. In the text, they themselves point out the many limitations and shortcomings of subjective performance tests and they stress that often the results can serve as a rough guide only. One wonders, however, whether or not it is wise to include some of the procedures of doubtful validity in a collection of methods that, coming from such an authoritative source, will be considered inevitably as a standard text. For the inexperienced, it is only too easy to forget all warnings and to accept the results of all the test methods without reservation.

At the present time, the performance of tinplate cannot be assessed completely by the physicist or the chemist, and much research, in which the authors are taking a prominent part, is going on to remedy this situation. It is hoped that future editions of this otherwise excellent booklet will be able to dispense eventually with all subjective test methods.

H. LIEBMANN

ÉLECTROCHIMIE THÉORIQUE. By EUGÈNE DARMOIS and GENEVIÉVE DARMOIS. Pp. viii + 239. Paris: Masson et Cie. 1960. Price 39 NF.

In this book the authors attempt in very few pages a survey of an extremely wide subject, and it is therefore inevitable that readers will be disappointed at omissions and curtailed treatment of important subjects. It is evident, however, that an endeavour has been made to say something about everything that should be included under the title of theoretical electrochemistry. Almost always, individual topics are discussed with clarity and direct logical sequence; specially good, although brief, treatments are given of ion mobility (chapter IV) and ion interaction theories (chapter VII). However, nothing but disappointment is engendered by less than one page on semi-conductors.

Of less value and significance are the discussions on electro-polishing, electro-reproduction and electro-plating in this book, where space could be devoted to expansion of more important topics.

The references to original work are particularly irritating, because without a bibliography they are useless, and much valuable space is lost by such interjections as "*Un de nos élèves, P. Charlon, s'est occupé de la question des* 1937."

Errors are very few; some confusion on sign convention for electrodes, the unfortunate use of G for ΔG and a drawing of a calomel electrode in which the platinum leading-in wire is not wholly immersed in the mercury are a few matters that need correction. The printing and binding are excellent, and the price is modest in these days of expensive text-books.

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Publications Received

PURE FOOD AND PURE FOOD LEGISLATION. Edited by A. J. AMOS, B.Sc., Ph.D., F.R.I.C. Pp. xii + 167. London: Butterworths Publications Ltd. 1960. Price 21s.
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Translated from Russian.

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X-RAY ABSORPTION AND EMISSION IN ANALYTICAL CHEMISTRY: SPECTROCHEMICAL ANALYSIS WITH X-RAYS. By H. A. LIEBHAFSKY, H. G. PFEIFFER, E. H. WINSLOW and P. D. ZEMANY. Pp. x + 357. New York and London: John Wiley & Sons Inc. 1960. Price $13.50; 108s.

ANALYTICAL CHEMISTRY OF THE RARE EARTHS. By R. C. VICKERY, D.Sc., Ph.D. Pp. viii + 139. Oxford, London, New York and Paris: Pergamon Press. 1961. Price 40s.

HANDBOOK OF TEXTILE TESTING AND QUALITY CONTROL. By ELLIOT B. GROVER and D. S. HAMBY. Pp. viii + 614. New York: Textile Book Publishers Inc. (A Division of Interscience Publishers Inc.). 1960. Price $17.50; 132s.

ORGANIC ELECTRONIC SPECTRAL DATA. Volume I, 1946–1952. Edited by MORTIMER J. KAMLET. Pp. xiv + 1208. New York and London: Interscience Publishers Inc. 1960. Price (single volume) $28.50; 215s.; (subscribers to whole series) $25.00; 188s.

ORGANIC ELECTRONIC SPECTRAL DATA. Volume II, 1953–1955. Edited by HERBERT E. UNGNADE. Pp. x + 919. New York and London: Interscience Publishers Inc. 1960. Price (single volume) $17.50; 132s.; (subscribers to whole series) $15.00; 113s.

COMPREHENSIVE ANALYTICAL CHEMISTRY. Volume IB. CLASSICAL ANALYSIS. Edited by CECIL L. WILSON, Ph.D., D.Sc., F.R.I.C., F.I.C.I., and DAVID W. WILSON, M.Sc., F.R.I.C. Pp. xxii + 878. Amsterdam, London, New York and Princeton: Elsevier Publishing Company; London: D. Van Nostrand Company Ltd. 1960. Price £7 15s.

A DICTIONARY OF NAMED EFFECTS AND LAWS IN CHEMISTRY, PHYSICS AND MATHEMATICS. By D. W. G. BALLENTYNE, B.Sc., Ph.D., A.Inst.P., and L. E. Q. WALKER, A.R.C.S. Second Edition. Pp. vi + 234. London: Chapman & Hall Ltd. 1961. Price 30s.

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